Pollination and breeding systems of alien invasive plants in KwaZulu-Natal in South Africa

by

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Thesis

Submitted in partial Fulfillment of the requirements

for the degree of Master of Science in the

School of Botany and Zoology

University of Natal

Pietermaritzburg

2001

Abstract

According to Baker (1955), success of plant invasions can be attributed to autogamous breeding systems and generalist pollination systems. A test of Baker's rule was carried out on 19 invasive alien plant species in KwaZulu-Natal, South Africa. Natural levels of fruit set in these plants was high (median= 71.5% fruit set per plant). Breeding system experiments for 18 species showed that 17% of the species are allogamous, 72 % are autogamous and 11% are apomictic. This contrasts markedly with a general flora of 1472 species in which 65% are allogamous, only 14% are autogamous, and 21% are apomictic. Because the breeding systems of the alien species were largely autogamous, pollinators make only a small contribution to the overall reproductive success. Seventy one percent of the 14 alien species for which pollinators were obtained were pollinated by honeybees, which are super-generalists and ubiquitous in a generalist pollination systems. However, some species showed pre-adaptation to relatively specialized pollination systems, involving hawkmoths, butterflies and *Xylocopa* bees respectively. Thus, alien plants were not visited by a wide range of insects, but rather showed pre-adaption to one or more pre-existing guilds in the new habitat. Pollen limitation experiments showed no evidence that reproductive success in populations of the species is limited by pollen availability.

Preface

The study was conducted from 1998 to 2000 in KwaZulu-Natal mainly around the Pietermaritzburg area, with one study site in Pinetown. Writing and data analysis was carried out in the School of Botany and Zoology, University of Natal. The study presented here is the original work by the author and where work by other authors has been mentioned the work has been duly acknowledged.

Acknowledgements

I thank Dr. Steven Johnson for his untiring support and supervision. I thank my research committee and Dr. Elisa Raulings for advice. All thanks to the postgraduate students in the ecological lab and the herbarium staff at the University of Natal Pietermaritzburg for their assistance. Financial assistance from the FRD (now NRF) is gratefully acknowledged.

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Chapter 1

Introduction

Background to the problem

The invasion of alien plants is as a worldwide environmental problem that potentially poses a threat to indigenous vegetation. Alien invasive plants are plants that spread into new areas away from areas of original introduction (Richardson *et al.* 2000). Whilst population expansion and long-distance colonization of plants is an integral part of natural ecosystems, it has been dramatically accelerated as humans have moved plants between continents or between ecosystems for thousands of years (Everett, 2000). In South Africa, much of the indigenous vegetation is heavily invaded by wide range of introduced plant species (Richardson *et al.* 1997). The number of alien invasive plant species, in KwaZulu Natal has arisen dramatically in recent years, and their concurrent impact on the indigenous vegetation in the region has reached an alarming level (Henderson, 1995).

Successful establishment of alien plant invasive has been attributed to their reproductive features that enable them to establish themselves successfully in the new environment (Baker, 1955). However, pollination and breeding systems of invasive alien plant species in South Africa has not been studied in detail. This study investigates (1) the relationship between breeding systems and success of alien invasive plant species and (2) the ability of alien invasive plant species to utilize pollinators in the adopted habitat.

Existing evidence for Baker's rule

Baker's rule (1955) states that the success of colonizing plants (e.g. weeds) is promoted by an autogamous breeding system or generalized pollination system. The reproductive output of a plant depends largely on the number of fruits and the number of seeds produced in them. It has been shown that flowers fertilized by pollen from a different plant (outcrossing) yield more capsules and more seeds than flowers fertilized by self-pollen (autogamy and geitonogamy) (Darwin, 1876). Self-

incompatible species are typically poor colonizers because two individuals are needed to start a population (Baker, 1967) (Table 1.1). However, various authors, such as Baker (1955), Stebbins (1957) and Carlquist (1966) have regarded the ability to self-fertilize as an important factor in colonizing species (Table 1.1), but Carlquist (1966) demonstrated that an outbreeding system is required to maximize variability in the progeny of invading species. Carlquist's (1966) argument was based on the high frequency (>40%) of dioecious species (separate male and female plants) in the Hawaiian Islands.

Hermaphroditic flowers (flowers with both male and female organs) presumably evolved to facilitate the deposition of outcross pollen and removal of pollen from anthers in a single visit by animal vectors (Gibbs & Bianchi, 1999). Hermaphroditism in flowers also increases the potential for self-pollination due to the close proximity of anthers and stigmas. Although there has been a lot of debate about the relative advantages of hermaphroditism over dioecy (Cockayne, 1958; Carlquist, 1966; Bawa 1980; Bawa, 1995; Culley *et al.* 1999), it is still unclear under which condition each strategy is optimal. In the absence of effective pollinators (and/ or other plants to mate with), dioecious and self-incompatible species will be clearly disadvantaged. However, if initial colonization is successful, the greater genetic variation of outcrossing species may prove to be an important competitive advantage (Carlquist, 1966).

Raven (1979) believed that immigration of *Oenothera* to South America species could have been made possible through the autogamous breeding system. Self-fertilization also provided reproductive assurance in the alien invasive tree species (*Miconia calvescens*) in Tahiti (South Pacific Ocean) (Meyer, 1998), because there is no need for pollen carriers to transport pollen to affect fertilization. However, self-pollination or autogamous breeding systems did not provide reproductive assurance in *Aquilegia canadensis* (Eckert & Schaefer, 1998). Ueckermann and van Rooyen (2000) found that the success of four weedy Namaqualand annuals in colonizing abandoned fields is not dependent on self-fertilization.

The evidence that autogamy provides reproductive assurance was derived from a range of studies that show relatively high levels of autogamy in colonizing plants (Levin, 1972; Price & Jain, 1981; Daehler, 1998; Panell & Barrett, 1998; Sun & Ritland, 1998). Baker's rule states that a self-compatible individual (Table 1.1) is more likely than a self-incompatible species (Table 1.1) to establish after long distance dispersal. Baker (1955) drew his conclusions from the documentation of self-compatible species in Oceanic Islands. Based on Baker's ideas, Stebbins (1957) later termed it "Baker's law". Baker (1967) re-instated what was termed "Baker's law" by Stebbins (1957) as a rule. Baker's rule thus emphasized the importance of reproductive success in colonizing species. Following Baker '(1955) principles, I tested the applicability of his rule on nineteen selected alien invasive species in South Africa.

Table 1.1. Breeding systems terminology and definitions

The terminology illustrates different concepts of breeding systems that have been used in this study:

- **Selfing** describes self-fertilization within a single flower (autogamy), or between flowers on the same plant (geitonogamy) as a result of pollinator movement (see Table 1.2).

- **Outcrossing** is fertilization between genetically different plants.

- **Self-compatibility** is a mechanism that allows self-pollen to fertilize the ovule.

- **Self-incompatibility** is a genetic mechanism that prevents self-fertilization from taking place.

Table.1.2. Relationship between plant mating patterns and dependency on pollinators

Mating pattern	Definition	Evidence	Dependency on pollinators
Apomixis	Seeds produced without fertilization	Fruit is set even when stigmas or anthers are removed[#]	Zero
Autogamy	Mechanism of self-fertilization within the flower itself	Fruit is set when pollinators are excluded	Very low
Allogamy	Selfing or outcrossing resulting from pollen transfer from a different flower	No fruit set when pollinators are excluded	High
Xenogamy	Obligate outcrossing, examples are, self-incompatible, dioecious and heterostylous species	Fruits is set only when flowers are outcrossed	Very high

Rare forms of apomixis that require pollination cannot be detected by conventional methods in this study (Richards, 1997).

Generalized versus specialized pollination systems

There is potential for alien invasive plants to suffer negative demographic effects when facing an entirely new guild of interacting pollinator species (Parker, 1997). It is therefore of importance to determine whether shortage of a suitable pollinator guild could limit the spread of aliens in the new

demographic environment. The prediction would be that species with generalized and flexible pollination systems should be more successful at invading new territory (Cox, 1989). Generalization versus specialization remains a critical issue in current studies of plant reproductive ecology (Johnson & Steiner, 2000). It has been suggested that long-lived species such as perennials will show more pollinator specialization because they can cope with reduced pollination service by opting for vegetative reproduction (Johnson & Steiner, 2000). However, annuals may be characterised by generalized pollination systems because they have to maximize seed production in order to spread rapidly.

Plants with specialized pollination systems should be less flexible in terms of attracting different pollinators in a new habitat. Yet flowers of garden plants are often pollinated by insects (very often honeybees) far removed in structure and behaviour from their native pollinators and the introduction of either pollinators or flowers into new environments may break down pollination specificity into a more generalized pollination system (Richards, 1997).

Aims and objectives

The aim of this study is to determine whether the invasion of alien plants into new environments can be attributed to breeding and pollination systems that result in high levels of seed production. Specifically the objectives of this study are to:

(1) document the levels of natural fruit and seed set in alien invasive plants in South Africa.

(2) experimentally determine the breeding systems of these species.

(2) study the degree of specialization versus generalization in pollination systems of alien invasive plants.

(3) determine if fruit and seed production of these species is limited by pollen availability.

(4) predict the invasive potential of species on the basis of their reproductive traits.

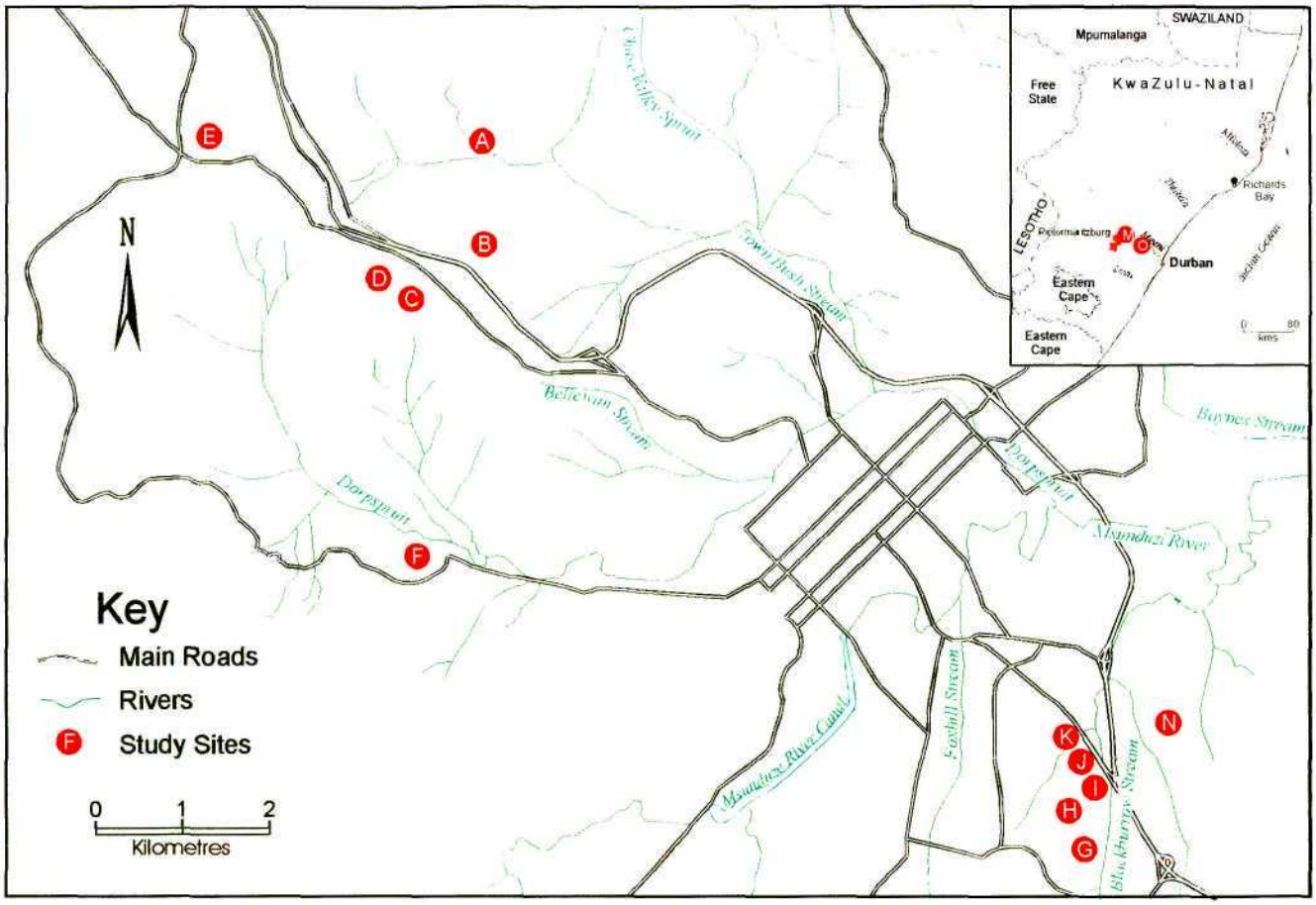
Introduction to the study species

Table.1.3. Alien invasive species selected for the study in Pietermaritzburg in South Africa. Species have been given rating of severity of invasion on the knowledge of their invasiveness throughout their distribution (in South Africa) from the literature ranging from severe, not severe or very severe, similar to the rating given by Caldwell (1997)(Henderson *et al*, 1987; Henderson, 1995; Caldwell, 1997).

Family	Species	Place of origin	Date of introduction	Severity of invasion in South Africa	Study sites
Asteraceae	*Ageratina adenophora*	Mexico & West Indies	1940	severe	Sweetwaters (PMB)
	Chromolaena odorata	Native to South & Central America	1940	very severe	Paradise Valley
Bignoniaceae	*Jacaranda mimosifolia*	South America	1888	not severe	Scottsville (PMB)
Cactaceae	*Opuntia stricta*	Central & South America	?	not severe	Ashburton
Cannaceae	*Canna indica*	West Indies & Central America	?	severe	Hilton
Convolvulaceae	*Ipomoea alba*	Tropical America	?	severe	Scottsville
Fabaceae	*Acacia mearnsii*	south-eastern Australia	1880	very severe	Ferncliffe
Fabaceae	*Caesalpinia decapetala*	Native to India & Sri Lanka	?	severe	Ferncliffe
Fabaceae	*Senna didymobotrya*	Tropical Africa	?	severe	Hayfields
Fabaceae	*Sesbania punicea*	South America	1960	severe	Scottsville
Liliaceae	*Lilium formosanum*	Asia (Taiwan)	?	not severe	World's View (PMB)
Meliaceae	*Melia azedarach*	south-western Asia	1894	not severe	Scottsville
Myrtaceae	*Eucalyptus camaldulensis*	Australia	?	severe	Ferncliffe
Myrtaceae	*Psidium guajava*	Mexico	?	severe	Scottsville
Onagraceae	*Oenothera biennis*	Central America	?	not severe	Hilton
Solanaceae	*Cestrum aurantiacum*	Guatemala	?	severe	Ferncliffe
Solanaceae	*Solanum mauritianum*	South America	Mid 19th century	very severe	Ferncliffe

Family	Species	Place of origin	Date of introduction	Severity of invasion in South Africa	Study sites
Zingiberaceae	*Hedychium gardnerianum*	Asia	?	not severe	World's View
Verbenaceae	*Lantana camara*	Central America	1858	very severe	Ferncliffe

Figure **1.1**. Map of Kwazulu-Natal in South Africa showing study sites around Pietermaritzburg in KwaZulu-Natal. Other study sites are found on the way to Durban (Table 1.4).



Study sites

Table 1.4. Co-ordinates represented by symbols of different study sites.

Symbol	Place	Species	Location
A	Ferncliffe	*Acacia mearnsii, Caesalpinia decapetala, Cestrum aurantiacum, Eucalyptus camaldulens, Lantana camara and Solanum mauritianum*	29° 33` 20``S 30° 19` 41`` E
B	Montrose	*Canna indica*	29° 34` 26`` S 30° 20` 00`` E
C	World's view	*Lilium formosanum* and *Hedychium gardnerianum*	29° 34` 53`` S 30° 19` 49`` E
D	Kettlefontein	*Hedychium flavum*	29° 34` 30`` S 30° 19` 15`` E
E	Hilton	*Oenothera biennis*	29° 33` 18`` S 30° 20` 02`` E
F	Sweetwaters	*Ageratina adenophora*	29° 36` 20`` S 30° 19` 41`` E
G	Epworth	*Sesbania punicea*	29° 37` 50`` S 30° 24` 21`` E
H	Scottsville	*Ipomoea alba*	29° 37` 44`` S 30° 24` 22`` E
I	Scottsville	*Melia azedarach*	29° 37` 42`` S 30° 24` 27`` E
J	Scottsville	*Jacaranda mimosifolia*	29° 37` 39`` S 30° 24` 24`` E
K	Scottsville	*Acacia multijuga*	29° 37` 36`` S 30° 24` 22`` E
L	Scottsville	*Psidium guajava*	29° 36` 22`` S 30° 23` 33`` E
M	Ashburton	*Opuntia stricta*	29° 41` 31`` S 30° 26` 52`` E
N	Hayfields	*Senna didymobotrya*	29° 37` 33`` S 30° 24` 39`` E
O	Paradise valley	*Chromolaena odorata*	29° 49` 42`` S 30° 50` 28`` E

Background to the study species

Nineteen alien invasive plant species were selected for this study because of their problematic spread in grasslands, riverine vegetation, forests and plantations. Voucher specimens are deposited in the Natal University Herbarium in Pietermaritzburg (NU). Species have been listed according to the family names.

Ageratina adenophora L. (Asteraceae)

Ageratina adenophora (Crofton weed) originates from Mexico and the West Indies. It is native to South America in the tropic regions (Caldwell, 1997). It was first recorded in the southwestern Cape in 1858 where it did not survive (Bromilow, 1995). It was reintroduced into South Africa around 1940's as a garden plant (Henderson *et al*, 1987). It is a multi-stemmed perennial herb or a softy woody shrub up to 2 m high. It grows along disturbed roadsides. It prefers a dry, moist habitat. The head-like inflorescence is a capitulum with several individual flowers called florets, arranged in the terminal of the branch forming corymbs; florets are ca. 70-80, white, receptacle glabrous, areolate; Ovary inferior; 5 stamens inserted into the corolla tube; fruit is one seeded, termed a cypsela. Flowers August-December. (Figure 1.4C).

Chromolaena odorata (L.) R.M. King and H. Robinson (Asteraceae: Eupatoriae) (*Sensu lato*)

This species is known as the triffid Weed or Paraffin weed in South Africa. The origin of its introduction is not well known, but it may have been introduced from Jamaica (Macfadyen & Skarratt, 1996) to other countries and spread through Asia from a plant in the Calcutta Botanical Gardens. It was first recorded in West Africa in the1930s (Macfadyen & Skarratt, 1996). It was first recorded in South Africa around 1940's in Durban (Pickworth, 1976). It is now cultivated as an ornamental plant (Henderson, 1995). It has become a major weed of crops, natural forests and plantations (Goodall & Erasmus, 1996). It is a scrambling, sparsely hairy shrub up to 4m high, often forming a dense thicket. It prefer moist habitat in disturbed sites. Terminal and axillary pedunculate corymbs, involucre cylindrical, 10 mm long, 3 mm in diameter, bracts straw-greenish coloured in five to six rows (Henderson *et* al. 1987); florets pale mauve-whitish, protruding from the involucre

(Henderson *et al.* 1987); stamens 5, not free; ovary inferior, one basal ovule; fruit is one-seeded. Flowers July-August.

Jacaranda mimosifolia D.Don (Bignoniaceae)

Commonly known as the Jacaranda Tree, this species was introduced from South America in 1888 (Henderson, 1995). Mainly used as an ornamental tree, this species is invading new regions of Ntatal and is presently classified as a potential weed. It grows in dry, moist areas along roadsides. It is a deciduous or semi-deciduous woody species up to 22 m high with a rounded spreading crown. The inflorescence is a panicle, produced in loose, pyramidal sprays; flowers are mauvish-blue; stamens 2; ovary superior, numerous ovules; fruits are broadly oval, flattish. Flowers September-November. (Fig. 1.4D).

Opuntia stricta (Haw.) Haw. (Cactaceae)

Commonly known as the Australian Pest Pear, this species originates from North and Central America (Henderson *et al.* 1987). It grows in disturbed dry habitat and was introduced as an ornamental plant but now it is spreading in dry savannas (Henderson *et al.* 1987). A spreading much-branched succulent shrub of 0.5-2m high. Flowers are solitary, sessile, yellow up to 70 mm long; stamens numerous; ovary inferior, ovules numerous; fruits are succulent berries, smooth and narrow towards the base, turning from red-purple. Flowers November-January (Fig. 1.2D).

Canna indica L. (Cannaceae)

This species is native to the West Indies and Central America (Heywood, 1996). It is a naturalised weed in South Africa. It has been used as an ornamental plant for greenhouses in temperate zones and in tropical gardens (Heywood, 1996). It is widespread in disturbed and dumping sites in subtropical areas (Heywood, 1996). Inflorescence is a raceme; petals red with orange spots; stamens form two series, the outermost series is composed of three petaloid staminodes and the inner series consist of one staminode and a free petaloid stamen with half an anther joined to it along the edge; ovary inferior, ovules≥20; fruit is a capsule. Flowers throughout the year.

Ipomoea alba L. (Convolvulaceae)

Ipomoea alba originates in tropical America. It grows in disturbed, moist habitats. It is a sub-woody, glabrous, annual or perennial climber. Flowers white, large, ± 140mm diameter, with a tube 70-120mm, open at dusk and withering, at dawn (Pooley, 1998); stamens 5; ovary superior, ovules 1-5; fruits are capsules, dehiscent. Flowers October-November (Fig. 1.3C).

Acacia mearnsii De Wild. (Fabaceae)

Acacia mearnsii (Black wattle) originates from southeastern Australia and was introduced into Natal for firewood (Henderson, 1995). Around 1880, the species was cultivated commercially for its bark (Caldwell, 1997). *Acacia mearnsii* is an evergreen tree 5-10 m high. It grows along roadsides and in plantations, prefers a dry, moist habitat. The inflorescence is spherical in shape and occurs at the end of branches; flowers are arranged in a large corymb, in fragrant sprays; the five petals are pale yellow- cream and fused to the apex; stamens ≥10; consist of polyad pollen (12 pollen grains); ovary inferior, ovules 5. Fruits are dark brown pods, markedly constricted; seeds are black, 5 mm long and 3.5 mm broad, elliptic, compressed, smooth, with a conspicuous caruncle. Flowers August-December.

Caesalpinia decapetala (Roth) Alston (Fabaceae)

Caesalpinia decapetala (Mauritius thorn) is a native of India and Sri Lanka. It grows in disturbed sites in dry, moist areas. It is a robust, woody, scrambling and prickly shrub to 2 to 4m or a climber up to 10m high. Inflorescence with flowers in dense axillary racemes, 10 inflorescences per plant with ± 30 flowers organised in cluster 100-400mm long; flowers pale yellow; stamens 10, free; ovary superior, ovules ≥ 10; fruits are green at an early stage of development and become brown at maturity (Henderson *et al.* 1987; Henderson, 1995; Caldwell, 1997). Flowers May-November. (Fig. 1.2A & B).

Senna didymobotrya (Fresen.) Irwin & Barneby (= *Cassia didymobotrya* Fresen.) (Fabaceae)
Commonly known as the Peanut Butter Cassia. *Senna didymobotrya* is native to tropical East Africa (Dulberger, 1981). It is a yellow flowering shrub grown as an ornamental in some parts of the world (Dulberger, 1981). It grows in dry moist areas. This species shows floral adaptations associated with buzz pollination. Numerous inflorescences occur on the same branch. The inflorescence is an erect raceme; open flowers 10-50; stamens 7, in three whorl, first whorl is composed of four stamens with filaments about 3 mm long and yellow anthers 4 mm long, the second whorl is a pair of large, lateral stamens having filaments ca. 3 mm long and anthers ca.11 mm long, thick, hard tapering twin structures, each with two basal appendages, curved, one inclining to the right and the other to the left, third whorl of stamens is a single type with a filament ca. 6 mm long and anther ca. 5 mm long; ovary superior, ovules ≥ 10. Flowers throughout the year, mainly April-August (Fig. 1.3D, E & F).

Sesbania punicea (Cav.) Benth. (Fabaceae)
Commonly known as the Red Sesbania. This species is native to eastern Argentina, southern Brazil and Uruguay (Henderson *et al.* 1987). It was introduced into South Africa as an ornamental plant (Logarzo & Casalinuovo, 1997). The species started spreading around Natal in 1960 and was proclaimed a weed in 1970 (Henderson *et al.* 1987). It is found growing along riverbanks. It is normally found growing along the riverbanks, as seeds are distributed mainly by water, but can also establish themselves in disturbed places such as roadsides or rubbish dumps (Henderson *et al.* 1987). It is a perennial, deciduous shrub 4-6m in height. Inflorescence a raceme up to 250 mm long; flowers orange-red, 20 mm long (Henderson *et al.* 1987; Caldwell, 1997); stamens 10; ovary superior, ovules numerous; fruit a pod, four-winged and 60-80mm long. Flowers October-December.

Lilium formosanum (B.K) (Liliaceae)
This is a perennial, monocotyledonous herb that originates from Asia (Wells *et al.* 1986). It is reported to be endemic to Taiwan (Hi Song, 1996) and grows mainly in disturbed areas. This species has been spreading into new sites in the last couple of years (Figure 1.2F). The inflorescence is a raceme with 1-4 flowers, white, ± 180 mm long; stamens 6; ovary superior, ovules numerous; fruits

are capsules, cylindrical, 5-8 cm long. Flowers January-April.

Melia azedarach L. (Meliaceae)

Commonly known as Syringa, this species originates from southwestern Asia (Bromilow, 1995). It is native to the areas extending from India to Australia (Caldwell, 1997). It was introduced into South Africa from India (Bromilow, 1995), and is now cultivated as an ornamental plant. It has reached the limits of its distribution in Natal, but can be expected to increase in abundance in the future (Caldwell, 1997). It grows in disturbed moist habitats. The inflorescence is a panicle; purple flowers are arranged in large, terminal, heavily -perfumed sprays; stamens 5-10 united into a staminal tube; ovary superior, ovules ≥ 2; Fruits green becoming yellow and wrinkled and persist until after the leaves fall. Flowers September-November.

Eucalyptus camaldulensis Dehnh. (Myrtaceae)

Commonly known as The Red River Gum, this species originates from southeastern Australia and is cultivated in Natal for timber, firewood and shelter (Henderson, 1995). It is an evergreen tree that can grow 5-10 m high. It grows in plantations and disturbed sites, in moist habitat. Inflorescence is a cyme; flowers cream; stamens numerous, exerted; ovary inferior, ovules numerous; fruits are reddish-brown capsules with prominent rims and protruding triangular valves. Flowers September-November. (Fig. 1.4B).

Psidium guajava L. (Myrtaceae)

Commonly known as Guava, *P. guajava* was introduced into South Africa from tropical America (Henderson, 1995). It grows in disturbed areas on abandoned agriculture fields. It was introduced as an agricultural crop (Bromilow, 1995). It is an evergreen shrub or small tree up to 5 m high. Inflorescence a cymose; flowers white; stamens numerous; ovary inferior, ovules ≥ 10; fruits many-seeded berries turning yellow with white, yellow or pink flesh. Flowers October-December.

Oenothera biennis L. (Onagraceae)

The evening primrose was introduced into southern Africa from Central America and it has been known as a weed since the end of the 19th century (Bromilow, 1995). It grows on dry moist habitat and is commonly found olong roadsides in waste areas (Bromilow, 1995). The inflorescence is a spike 40 cm long; flowers are ± 60 mm in diameter, yellow, opening at sunset and closing in the morning; stamens 8; fruits capsule, 20-30 mm long, seeds ≥ 200. Flowers January-April. (Fig. 1.2D & C).

Cestrum aurantiacum Lindl. (Solanaceae)

Cestrum aurantiacum (Yellow or Orange Cestrum) originates from Guatemala (Henderson *et al.* 1987). The plant is now spreading into plantations and disturbed sites. It grows in dry, moist habitat. It is a branched, evergreen shrub of 1-2m high. The inflorescence is typically an axillary cyme; flowers occur in groups of two, sessile, petals orange-yellow, 20-25 mm long, the large acute lobes strongly reflexed (Henderson *et al.* 1987); stamens 5; ovary superior with numerous axile ovules; fruits small, white globose spongy berries (Henderson *et al.* 1987; Henderson, 1995; Caldwell, 1997). Flowers May-August. It is grown as an ornamental plant in South Africa (Fig. 1.2A).

Solanum mauritianum Scop. (Solanaceae)

Commonly called Bugweed. This species was originally thought to be indigenous to tropical Asia, but has now been discovered to be indigenous to South America (Wells *et al.* 1986; Bromilow, 1995). It was introduced into South Africa in the mid 19th century (Denny & Goodall, 1992). The weed invades natural areas, and plantations (Campbell *et al.* 1992). It prefers a dry moist habitat. The mode of pollination with this type of floral structure is referred to as buzz pollination (Buchmann, 1983) (Fig. 1.3A & B). The shrub branches extensively into a small tree. The inflorescence is a combination of cymes; flowers are purple in compact terminal clusters on a felty stalk (Henderson, 1995); stamens 5; ovary superior, ovules≥ 10; fruits globose 10mm in diameter and surrounded at the base by persistent calyces. Flowers throughout the year.

Hedychium gardnerianum Roscoe. (Zingiberaceae)

This species originates in Asia and was introduced as an ornamental herb into South Africa (Henderson, 1995). This species invades forests, plantations, riverbanks and shaded areas (Henderson, 1995). It grows in a moist, disturbed habitat. It is a robust, rhizomatous herb up to 2m. The inflorescence occurs as a dense raceme; flowers showy, slender tube with long-exerted stamen, one fertile stamen; ovary inferior, ovules≥10. Flowers January-March.

Lantana camara L. (Verbenaceae)

Commonly known as Lantana, this species originates from tropical Central America (Henderson *et al.* 1987), and was introduced into South Africa from India (Pooley, 1998). *Lantana camara* is a shrub that is 1 to 3m tall. It is possibly the most serious alien plant invader in South Africa. It grows vigorously in a wide variety of environmental conditions, but thrives better under humid conditions (Baars & Neser, 1999). The inflorescence is cymose; flowers irregular, with different colours; two pairs of stamens are attached to the corolla at different heights; anthers and stigma are inserted and the stigma lies below the lower pair of anthers; ovary superior, bilocular, and bears one ovule in each locule(Mathur & Mohan Ram, 1986); fruits are fleshy drupes, glossy green turning purplish-black when they are ripe (Henderson, 1995). It flowers throughout the year in moist, frost-free areas. The flower in the centre of the inflorescence opens first and usually changes colour as it fades, and undergo colour changes from yellow to orange and from orange to red in a 24-hour period (Schemske, 1976; Mathur & Mohan Ram, 1986), the yellow flowers are attractive to butterflies and offer nectar and have receptive stigma (Dronamraju, 1958; Weiss, 1991; Weiss, 1995).

Figure **1.2**. Floral morphology and pollinators of alien plants: **A**. Inflorescence of *Caesalpinia decapetala*. Bar = 12.5 mm. **B**. Honeybee (*Apis mellifera*) robbing nectar from the base of the flower of *Caesalpinia decapetala*. Note 10 protruded anthers. Bar = 3 mm. **C**. Flower of *Ipomoea alba* are solitary. Bar = 15 mm. **D**. *Oenothera biennis* showing inflor escence with buds and opened flowers. Bar = 12.5 mm. **E**. Flowers of *Oenothera biennis* have four yellow petals, four anthers and four spreading stigmatic lobes. Bar = 7 mm. **F**. Habitat of *Lilium formosanum*. Bar = 100 mm. (Photographs by S.D. Johnson)


A
B
C
D
E
F

Figure **1.3**. Floral morphology and pollinators of alien plants: **A**. Floral structure of *Solanum mauritianum* showing poricidal anthers. Bar = 4 mm. **B**. Honeybee (*Apis mellifera*) pollinating on *Solanum mauritianum*. Bar = 6 mm. **C**. *Xylocopa flavorufa* buzz pollinating on *Senna didymobotrya*. Bar = 10 mm. D. Floral morphology of *S. didymobotrya* showing 7 stamens. Bar = 4 mm. **E**. Bagging experiment on the flowers of *Senna didymobotrya*. Bar = 19.5 mm. **F**. Inflorescences of *S. didymobotrya*. Bar = 18.2mm. (Photographs by S.D. Johnson, except **E**. by T.D. Rambuda)


B


D


F

Figure **1.4**. Floral morphology of alien plants: **A**. Inflorescence of *Cestrum auritiacum*. Bar = 15 mm. **B**. Flowers of *Eucalyptus camaldulensis* and honeybee (*Apis mellifera*) pollinating the cream-white flowers. Bar = 20mm. **C**. Inflorescence of *Ageratina adenophora* is a capitulum with several individual florets. Bar = 15mm. **D**. Bagging experiment on *Jacaranda mimosifolia*. Bar = 50 mm. (Photographs by T.D. Rambuda).


A
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Chapter 2

Breeding systems of alien invasive plants in South Africa

Abstract

According to Baker's (1955) rule, plants capable of uniparental reproduction, such as self-compatible species are more likely to establish after long distance dispersal than self-incompatible or dioecious species. Breeding system experiments showed that 72% of 18 sampled alien invasive plants in South Africa are autogamous, while 11% are apomictic. This differs significantly from a general flora in which only 14% of species were autogamous and 21% were apomictic. These results suggest that invasion of alien plants is promoted by autogamy.

Introduction

Colonizing plants may suffer a pollination deficiency in their new habitat, either because conspecifics are not available for mating, or because pollinators to which the plants are adapted are not present. The breeding system of an alien invasive plant may be an important factor affecting the reproductive success of that plant (Lloyd, 1992, Santandreu & Lloret, 1999), and the ability to self-fertilize may be important for the successful establishment of plants in new areas which suffer a pollination deficiency (Baker, 1955).

Darwin (1876) questioned the advantage of self-fertilization in plants, when the progeny produced are often less vigorous than those of plants that are cross-fertilized (Holsinger *et al.* 1984). Stebbins (1957) proposed that the breeding system of a plant is a compromise between the conflicting demands of immediate versus long-term fitness. The ecological conditions and fitness pressures which a plant experience may determine whether outcrossing or self-fertilizing is the optimal breeding strategy in that environment (Stebbins, 1957). Breeding system experiments have been previously conducted in a wide range of species (Lloyd, 1992), but there has been no systematic

attempt to document the breeding systems of a weed flora. Therefore, it is of interest to compare the breeding systems of alien invasive plants with the breeding systems of the general flora in South Africa, to test the prediction that self-fertilization promotes initial colonization in new habitats (Baker, 1955). The aim of this chapter is to determine whether the invasion of alien plant species into new habitats is associated with different types of breeding systems.

Previous studies of plant mating have used enzyme electrophoresis to give measures of outcrossing versus selfing (Barret & Husband, 1990). Enzyme electrophoresis on the other hand provides a good measure of the *realized* mating system, as a consequence of plant breeding systems, population size and pollinator behaviour (Barrett & Husband, 1990). However, in this study classical bagging experiments (in which bagged flowers are hand pollinated) were used to determine whether the plants are apomictic, autogamous, self-compatible and allogamous, or xenogamous (Table 1.2). Classical bagging experiments are valuable for showing the ability of a plant to set seeds in the absence of pollinators, and to determine the degree of self-compatibility. Bagging experiments were used in preference to enzyme electrophoresis because the aim was to document the dependency of alien invasive species on pollinators, rather than the actual selfing rates.

Methods and Materials

Breeding system experiments were conducted on 18 alien invasive species (Table 1.3) during 1998, 1999 and 2000. Pollinators were excluded from flowers by bagging inflorescences with nylon mosquito-netting bags. Flowers were marked with coloured strings for different treatments. Plantex was smeared onto the inflorescence stalks of *Lantana camara* and *Solanum mauritianum* to prevent crawling insects, such as thrips from pollinating flowers inside the bags. On each inflorescence, flowers were cross-pollinated, self-pollinated or remain unmanipulated to flower development inside the bag to test for autogamy. The control treatment involves exposing the flowers to open pollination and waiting for fruit set without any manipulation of the flower development. Each treatment was applied to a number of flowers randomly selected per species, flowers were selected from each

individual plant; e.g. 12-20 flowers when testing for autogamy, self-pollination and outcrossing. In some species buds were emasculated to test for apomixis. The flowers of Asteraceae have the anthers fused in a tube around the stigma, and the anthers shed pollen before the stigma is receptive (Protandry). To ensure the stigma was removed early enough to avoid selfing, the stigmas and anthers were removed while the capitulum was still in bud. Pollen for the outcrossing treatment was collected of at least 10 meters away from the experimental plant, to avoid cross-pollination between two closely related plants (Waser & Price, 1989). To test for self-compatibility, flowers were pollinated by pollen from anthers on the same inflorescence. Pollen was collected by rubbing a toothpick against the dehiscent anthers of donor flowers and transferred immediately to the recipient stigma (Waser & Price, 1989). To test for autogamy, flowers were left in bags and not manipulated in any way.

The number of fruits set and the number of seeds per fruit were recorded for each treatment. Fruit set was recorded as present or absent for each flower in each treatment. Percentage fruit set was calculated by dividing the number of fruit set by the total number of treated flowers ($n \geq 12$) as a percentage. Flowers that did not set fruit were not used when calculating the mean number of seeds per fruit. Seed and fruit mass were measured as this may reflect on seed quality.

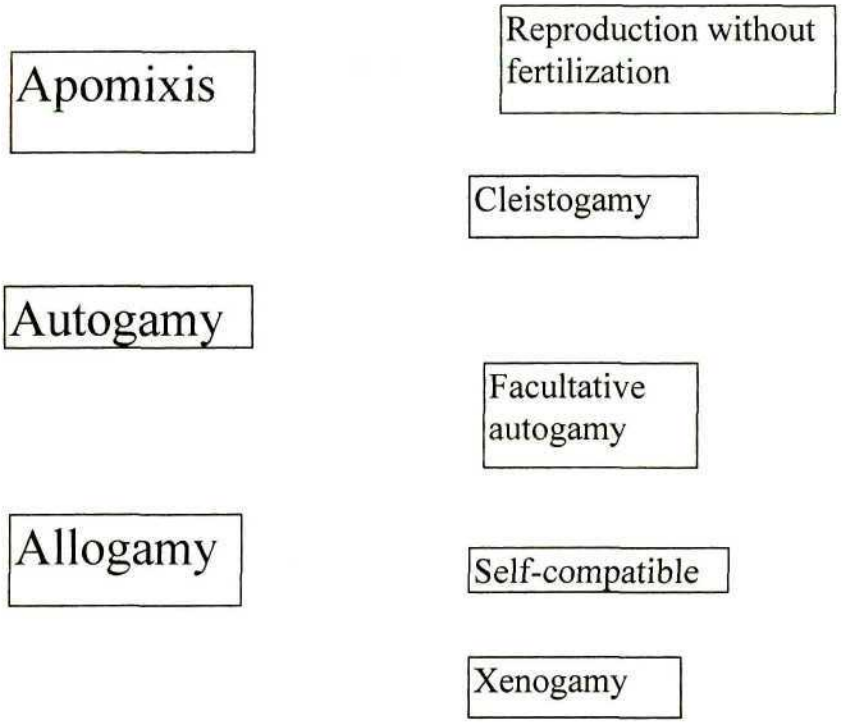

Figure **2.5**. Schematic outline of plant breeding systems, following Cruden (1976). Allogamy includes all species that are not autogamous. Xenogamy includes self-incompatible or dioecious species, and is a form of allogamy.

Data analysis

Fruit set was analysed using the chi-square test (Zar, 1996). The difference in seed set, fruit and seed mass between treatments was analysed using one-way ANOVA, followed by the Tukey multiple range test to see where significant differences lie (Zar, 1996). A plant is considered autogamous when there was no significant difference in fruit and seed set between unmanipulated and hand-pollinated treatments. A plant is self-compatible when there is no significant difference between selfed and outcrossed treatments. Self-incompatibility occurs when there is little or no seed set in self-pollinated plants.

Results

Table 2.5. Breeding systems results listed according to the family names in the alphabetical list in table 1.3.

✔ = type of breeding system.

Family	Species	Apomictic	Autogamy	Self-compatible	Self-incompatible
Asteraceae	*Ageratina adenophora*	✔			
Asteraceae	*Chromolaena odorata*	✔			
Cactaceae	*Opuntia stricta*		✔		
Cannaceae	*Canna indica*		✔		
Convolvulaceae	*Ipomoea alba*		✔		
Fabaceae	*Acacia mearnsii*		✔		
Fabaceae	*Caesalpinia decapetala*		✔		
Fabaceae	*Senna didymobotrya*			✔	
Fabaceae	*Sesbania punicea*		✔		
Liliaceae	*Lilium formosanum*		✔		
Meliaceae	*Melia azedarach*		✔		
Myrtaceae	*Eucalyptus camaldulensis*		✔		
Myrtaceae	*Psidium guajava*		✔		
Onagraceae	*Oenothera biennis*			✔	
Solanaceae	*Cestrum aurantiacum*		✔		
Solanaceae	*Solanum mauritianum*			✔	
Zingiberaceae	*Hedychium gardnerianum*		✔		
Verbanaceae	*Lantana camara*		✔		
Total	18	2	13	3	0

Results in table 2.5 represent eighteen alien invasive plant species whilst there were nineteen alien invasive plant species tested for breeding systems, this is because the results for *Jacaranda*

mimosifolia were not included, as they did not show any conclusive results. Autogamy and apomixis is the general trend among 18 alien invasive species (Table 2.5). Thirteen species are autogamous, three apomictic and three self-compatible.

Ageratina adenophora (Asteraceae)

Ageratina adenophora has florets arranged within a capitulum. The maximum number of fruits produced per capitulum in this study was 60 fruits per capitulum. Results show that *Ageratina adenophora* is apomictic. There was no significant difference in percentage fruit set between apomixis, autogamy and open pollinated florets (Figure 2.6 A). There was 80% fruit set in apomixis (AP) and autogamy (AU) compared to 90 % fruit set in the open pollinated (OP) treatment. However, there was a significant difference in the number of fruit per capitulum in the apomixis compared to the open pollinated and autogamy treatments (Fig. 2.6 B).

Chromolaena odorata (Asteraceae)

Chromolaena odorata also has florets arranged within a capitulum. The maximum number of fruits per capitulum in this study was 20 fruits per capitulum. Results suggest the breeding system of *Chromolaena odorata* is apomictic. There was no significant difference in percentage fruit set between apomixis, autogamy and open pollinated florets (Fig. 2.7 A). Unmanipulated and open pollinated florets set 99 % fruit as compared to 95 % in the apomixis treatment. There was no significant difference in the number of fruits per capitulum in the apomixis treatment compared to the open pollinated and autogamy treatments (Fig. 2.7 B).

Jacaranda mimosifolia (Bignoniaceae)

Bagged flowers produced no fruit after treatments of selfed and outcrossing were conducted. Twenty flowers tested for autogamy produced no fruit. Honeybees were observed visiting the flowers, which suggests the possibility of outcrossing. However, a low pollen count showed that honeybees might be poor pollinators (it will be discussed in Chapter 3). Many flowers produced by this species appear to be aborted and low levels of natural fruit set were recorded in this species (Fig. 4.27).

Opuntia stricta (Cactaceae)

The maximum seed set of *Opuntia stricta* in this study was 79 seeds per fruit. There was no significant difference in fruit set between the bagged and open-pollinated treatments (Fig. 2.8 A). There was 93% fruit set in the autogamy treatment compared to 100% in the open pollinated treatment. Fruit mass (Fig. 2.8 B) and seed mass per fruit crop (Fig. 2.8C) did not differ significantly between bagged and open pollinated flowers. The average number of seeds per fruit was 43-63, but the number of seeds produced did not differ significantly between treatments (Fig. 2.8D), which indicates that this species is autogamous.

Canna indica (Cannaceae)

Maximum seed set of *Canna indica* in this study was 19 seeds per fruit. Percentage fruit set was not significantly different between autogamy, selfed and outcrossing treatments (Fig. 2.9A). There was 90% fruit set in the autogamy treatment compared to 74 % for outcrossing treatment and 64% fruit set for selfed, indicating that the breeding system of this species is autogamous. The mass of fruit (Fig. 2.9B) and the total seed mass per fruit (Fig. 2.9C) were not significantly different between treatments. The average number of seeds per fruit was between 6-10, and did not differ between treatments (Fig. 2.9 D).

Ipomoea alba (Convolvulaceae)

The maximum seed set of *Ipomoea alba* in this study was four seeds per fruit. Percentage fruit set for autogamy, selfed and outcrossing treatments did not differ significantly (Fig. 2.10A). There was a significantly higher percentage fruit set in the outcrossing treatment (58%) compared to selfed (42%) and autogamy (42%) treatments. However, there was no significant difference in the fruit mass between treatments (Fig. 2.10 B), total seed mass per fruit (Fig. 2.10C) or the number of seeds per fruit (Fig. 2.10D), indicating an autogamous breeding system.

Acacia mearnsii (Fabaceae)

The Mimosoideae are characterised by having polyad pollen, and *A. mearnsii* has 12 pollen grains per polyad (personal observation). The maximum seed set of *Acacia mearnsii* in this study was seven seeds per fruit. Percentage fruit set was not significantly different between autogamy and open pollinated treatment (Fig. 2.11A). Fruit set in the autogamy treatment (52%) was not significant different to open pollinated flowers (75%), which indicates an autogamous breeding system in this species. The mass of fruit (Fig. 2.11B) was not significantly different between treatments (Fig. 2. 11 B). However, there was a significant difference in individual seed mass (Fig. 2.11C) and the number of seeds per fruit between the two treatments (Fig. 2.11 D). The discrepancy between the polyad pollen (12 pollen grains) and the low seed set for both open-pollinated and autogamous treatments may be a result of resource limitation, pollen quality or adhesion.

Caesalpinia decapetala (Fabaceae)

Caesalpinia decapetala showed a maximum of seven seeds per fruit in this study. Percentage fruit set was not significantly different for the autogamy treatment (35%), selfed (38%) and outcrossing treatments (99%) (Fig. 2.12A), indicating that the species is autogamous. Fruit mass was significantly lower in the autogamy treatment as compared to selfed and outcrossing treatments (Fig. 2.12 B). However, there was no significant different in the total seed mass per fruit and the number of seeds produced per fruit (Fig. 2.12 C&D).

Senna didymobotrya (Fabaceae)

The maximum seed set of *Senna didymobotrya* in this study was 10 seeds per fruit. Percentage fruit set was significantly lower in the autogamy treatment (13%) than in the selfed (53%) and outcrossed (60%) treatments (Fig. 2.13 A). This species is self-compatible, but not autogamous. The fruit mass and total seed mass per fruit was lower in the autogamy treatment compared to the selfed and outcrossed treatments (Figure 2.13 B & C). The average number of seeds per fruit varies between 2-5. There was lower number of seeds in the autogamy treatment compared to self-pollination and outcrossing treatments (Fig. 2.13D). Unfortunately, very few individuals set fruit in the autogamy

treatment (n = 2), so the sample size for fruit mass, total seed mass per fruit and the number of seeds per fruit is too low for significance testing. This experiment would ideally be repeated with a larger sample size in order to test the observed trends.

Sesbania punicea (Fabaceae)

The maximum seed set of *Sesbania punicea* in this study was six seeds per fruit. Percentage fruit set was not significantly different for the three treatments (Fig. 2.14A). There was 36% fruit set in the autogamy treatment compared to 57% for both selfed and outcrossing treatments, which indicates an autogamous breeding system in this species. The mass of fruit and total seed mass per fruit was not significantly different between treatments (Fig. 2.14 B & C). The average number of seeds per fruit was 3-4, and the number of seeds produced did not differ between treatments (Fig. 2.14 D).

Lilium formosanum (Liliaceae)

Lilium formosanum produced a maximum potential of 1470 seeds per fruit in this study. There was no significant difference in percentage fruit set between the three treatments (Fig. 2.15 A). There was 75% fruit set in the autogamy treatment compared to 92% for both selfed and outcrossing. This species is autogamous. There was no significant difference in fruit mass and total seed mass per fruit (Fig. 2.15 B & C). The average number of seeds produced per fruit is 800-1000 (Fig. 2.15 D). There was no significant difference in seed set between treatments (Fig. 2.15 D).

Melia azedarach (Meliaceae)

Breeding systems results in 1998 did not show any conclusive results. There was no fruit set observed in both treatments. Breeding systems experiments in 1999 were interrupted by rain. However, fruit set in bagged flowers (25% fruit set, n=12) was comparable to the open-pollinated controls, suggesting autogamy.

Eucalyptus camaldulensis (Myrtaceae)

The maximum seed set of *Eucalyptus camaldulensis* in this study was 150 seeds per fruit. Percentage fruit set was not significantly different for the three treatments (Fig. 2.16A). In the autogamy treatment there was 92% fruit set compared to 100% for both selfed and outcrossing treatments, indicating an autogamous breeding system. Fruit mass was not significantly different between treatments (Fig. 2.16 B). However there was significantly higher total seed mass per fruit in outcrossing treatment compared to autogamy and selfed treatments (Fig. 2.16C). The average number of seeds produced per fruit, was 60-80 and did not differ between treatments (Fig. 2.16 D).

Psidium guajava (Myrtaceae)

The maximum seed set of *Psidium guajava* in this study was 65 seeds per fruit. There was no significant difference in percentage fruit set between autogamy and open pollinated treatments (Fig. 2.17). In the autogamy treatment there was 93% fruit set compared to 100% in the open pollinated treatment, which indicates an autogamous breeding system. There was also no significant difference in fruit mass or total seed mass per fruit between treatments (Fig. 2.17 B & C). However, there was a significant difference in the number of seeds produced per fruit between treatments (Fig. 2.2.17 D), open pollinated treatment set more seeds per fruit than the autogamy treatment.

Oenothera biennis (Onagraceae)

The maximum seed set of *Oenothera biennis* in this study was 413 seeds per fruit. Fruit set in the selfed and outcrossed treatments was significantly more than double the fruit set in the autogamy treatment (Fig. 2.18 A). In the autogamy treatment there was 29 % fruit set compared to 67% in the selfed treatment and 83% in the outcrossing treatment. This shows that this species is self-compatible and weakly autogamous. There was no significant difference in fruit mass between treatments (Fig. 2.18 B). Seed mass per fruit was significantly lower in the autogamy treatment than in the selfed and outcrossed treatments (Fig. 2.18 C). Seed set per fruit differed significantly between the autogamy and selfed and outcrossing treatments (Fig. 2.18 D).

Failure to set fruit in the autogamy treatment in 1998 suggested that *O. biennis* was not autogamous. However, in a second experiment in 1999 (shown here), there was a substantial increase in fruit set in all treatments as compared to the previous year and roughly 20% of flowers in the autogamy treatment produced fruits (Fig. 2.18 A). High fruit set after self-pollination convincingly showed self-compatibility in *O. biennis*. In 1998 the problem was with the technique used, the bags for bagging flowers were to big for the flowers to allow proper development of the fruit as they quickly collapses during rain and reduce the volume of the bag compared to the medium size bags used in 1999.

Cestrum aurantiacum (Solanaceae)

The maximum seed set of *Cestrum aurantiacum* in this study was four seed per fruit. There was a significant difference in percentage fruit set between bagged and open-pollinated flowers (Fig. 2.19 A). Bagged flowers set 67% fruit compared to 88% in the open pollinated treatment. There was significantly higher fruit mass in open pollinated flowers compared to bagged flowers (Fig. 2.19 B). Seed mass did not differ significantly between treatments (Fig. 2.19C). The average number of seeds set was 2-4, and did not differ significantly between bagged and open-pollinated flowers (Fig. 2.19 D), which indicates an autogamous breeding system.

Solanum mauritianum (Solanaceae)

The maximum number of seeds produced by *Solanum mauritianum* in this study was 208 per fruit. Fruit set did take place in the autogamy treatment, although it was significantly lower than in the selfed and outcrossing treatments (Fig. 2.20 A), which indicate that *Solanum mauritianum* is self-compatible and partially autogamous. There was 34% fruit set in the autogamy treatment compared to 86% in selfed treatment and 76% in outcrossing treatment. However, there was no significant difference in fruit mass, seed mass and number of seeds per fruit between treatments (Fig. 2.20 B, C & D).

Hedychium gardnerianum (Zingiberaceae)

The maximum seed set of *Hedychium gardnerianum* in this study was 11 seeds per fruit. Percentage fruit set was not significantly different for autogamy, selfed and outcrossing treatments (Fig. 2.21A). There was 100% fruit set for both autogamy, selfed and outcrossing, indicating an autogamous breeding system. Fruit and seed mass were not significantly different between treatments (Fig. 2.21 B & C). Average seed set per fruit is ca. six and there was no significant difference in the number of fruit produced for each treatment.

Lantana camara (Verbanaceae)

The maximum seed set of *Lantana camara* in this study was not determined; success in reproduction was measured in fruit set per inflorescence. Percentage fruit set was significantly lower in the selfed treatment compared to autogamy and outcrossing (Fig. 2.22 A). In both the autogamy and outcrossing treatments there was 50% fruit set as compared to 29% in the selfed treatment, indicating an autogamous breeding system. Fruit mass and number of fruit per inflorescence were not significantly different between treatments (Fig. 2.22 B&C).

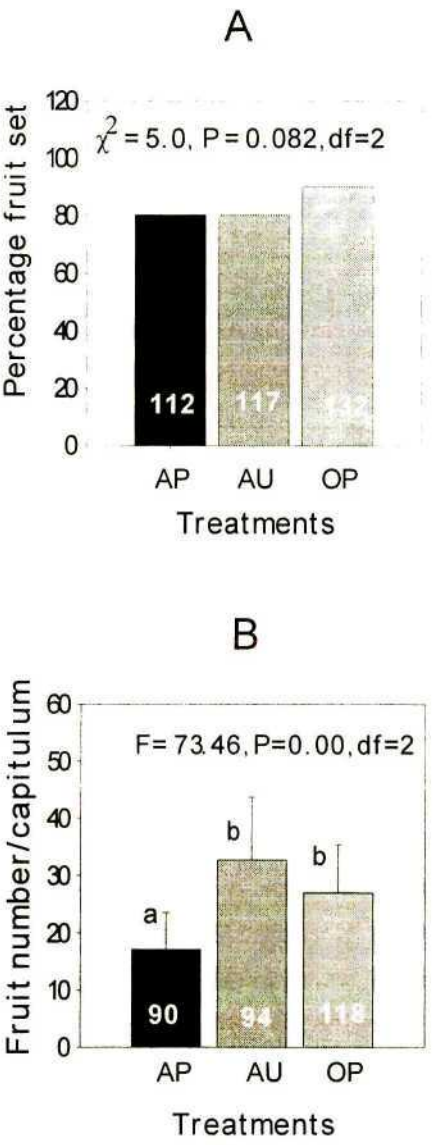

Figure 2.6. Breeding systems results for ***Ageratina adenophora***. A. Percentage fruit set (n=number of capitulum). B. Number of fruit (n=number of capitulum). AP=Apomixis, AU= Autogamy and O P = Open pollination. Bars with the same symbol are not significant from each other. Error bars =standard deviation.

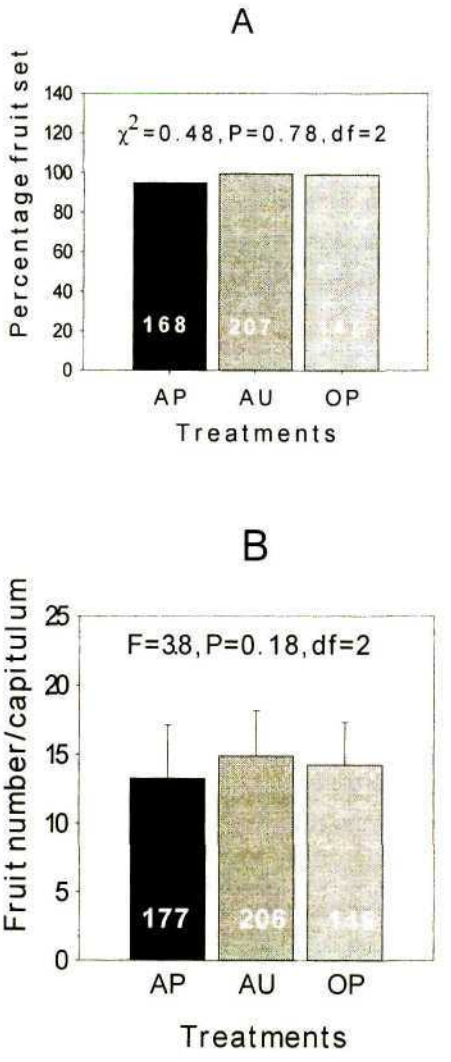

Figure 2.7. Breeding systems results for ***Chromolaena odorata***. A. Percentage fruit set (n= number of capitulum). B. Number of seeds (n=number of capitulum). AP =Apomixis, AU= Autogamy and OP=Open pollination. Error bars represent standard deviations.

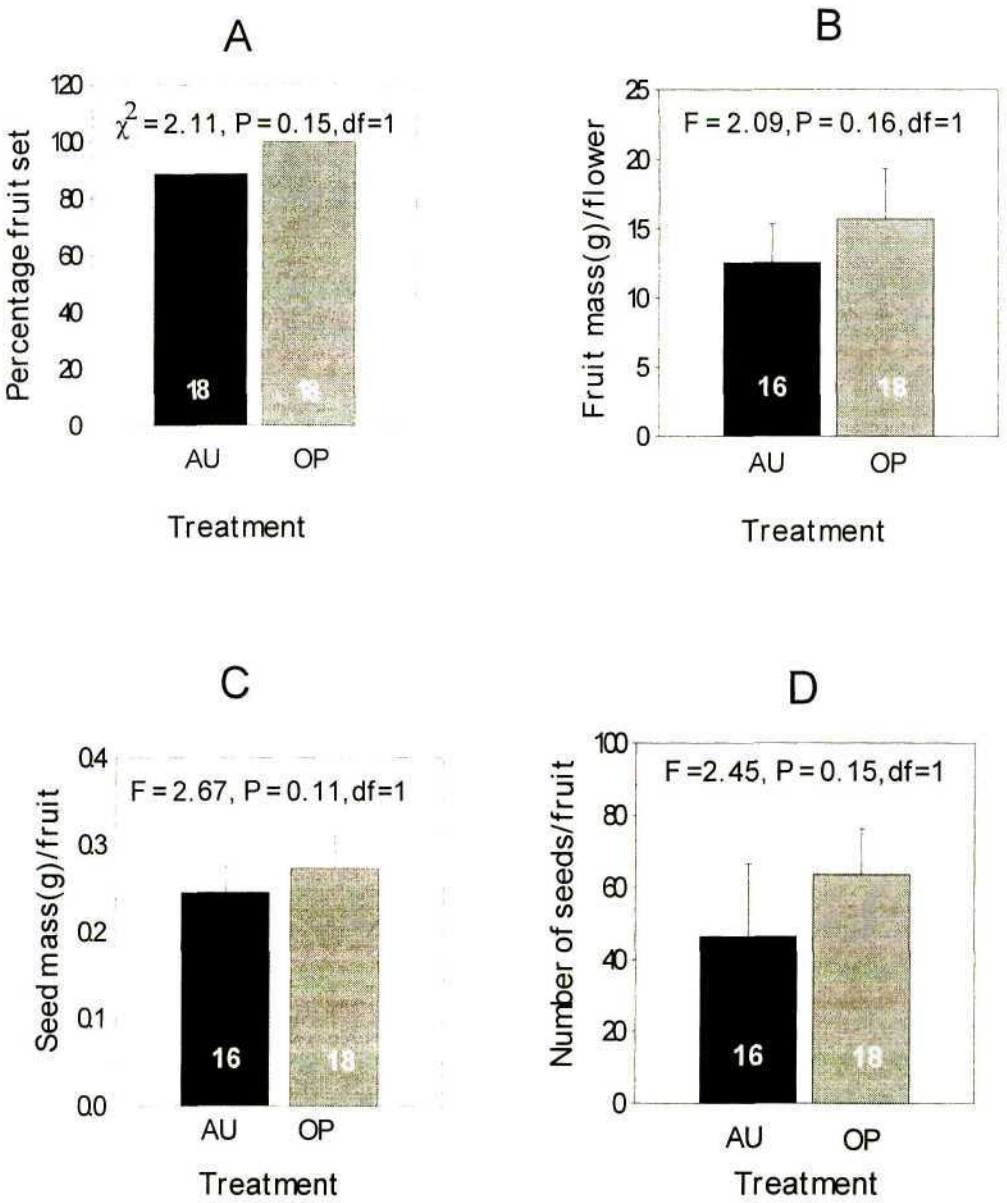

Figure 2.8. Breeding systems results for ***Opuntia stricta***. A. Percentage fruit set (n =number of flowers). B. Fruit mass (g) (n=number of fruits). C. Seed mass (g) (n=number of fruits). D. Number of seeds per fruit (n=number of fruits). AU=Autogamy, OP=Open (unbagged) pollinated flowers. Error bars represent the standard deviation.

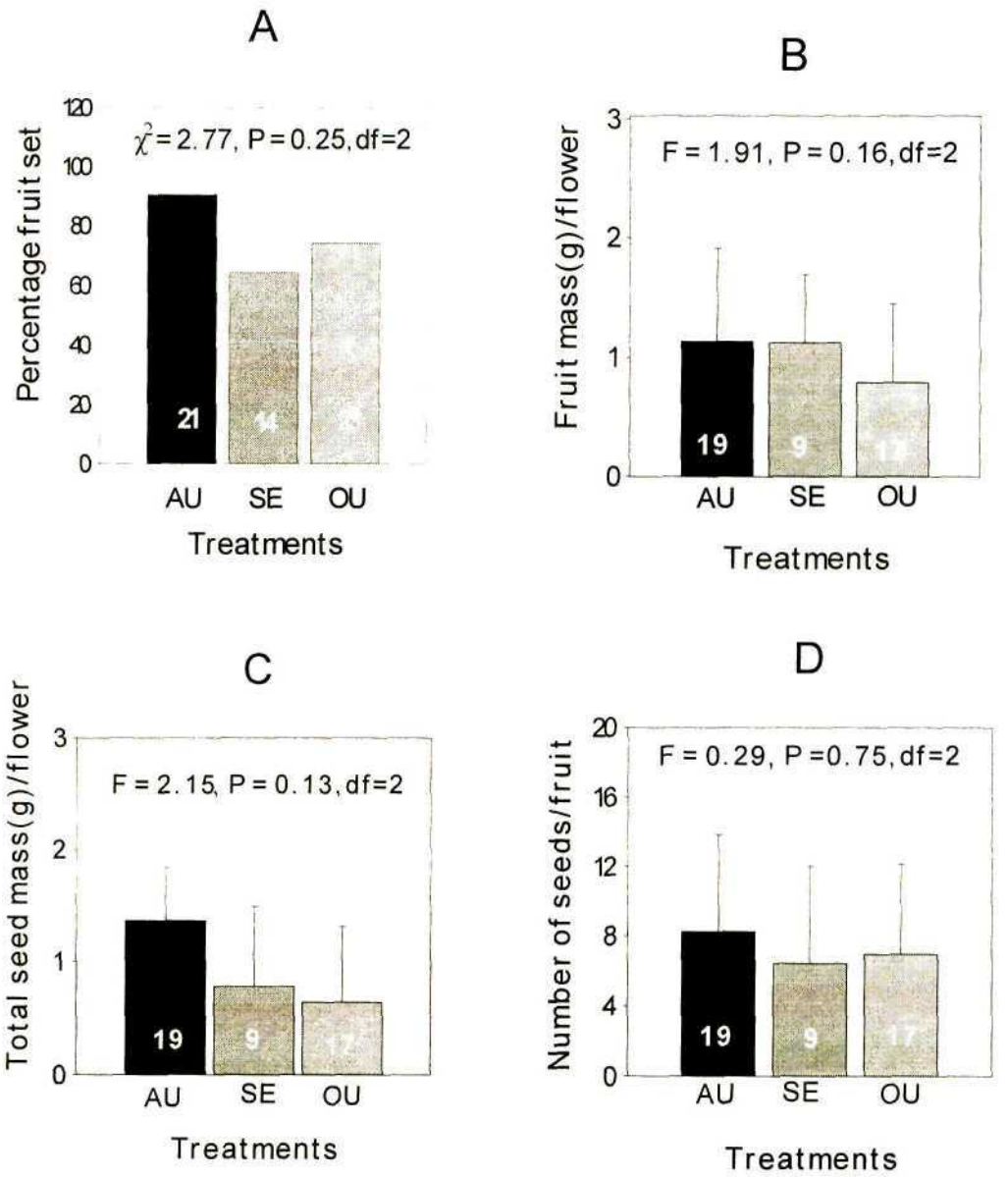

Figure 2.9. Breeding systems results for ***Canna indica***. A. Percentage fruit set (n=number of flowers). B. Fruit mass (g) (n=number of fruits). C. Seed mass (g)(n=number of seeds). D. Number of seeds per fruit (n=number of fruits). AU=Autogamy, SE=Self-pollination and OU=Outcrossing. Error bars= standard deviation.

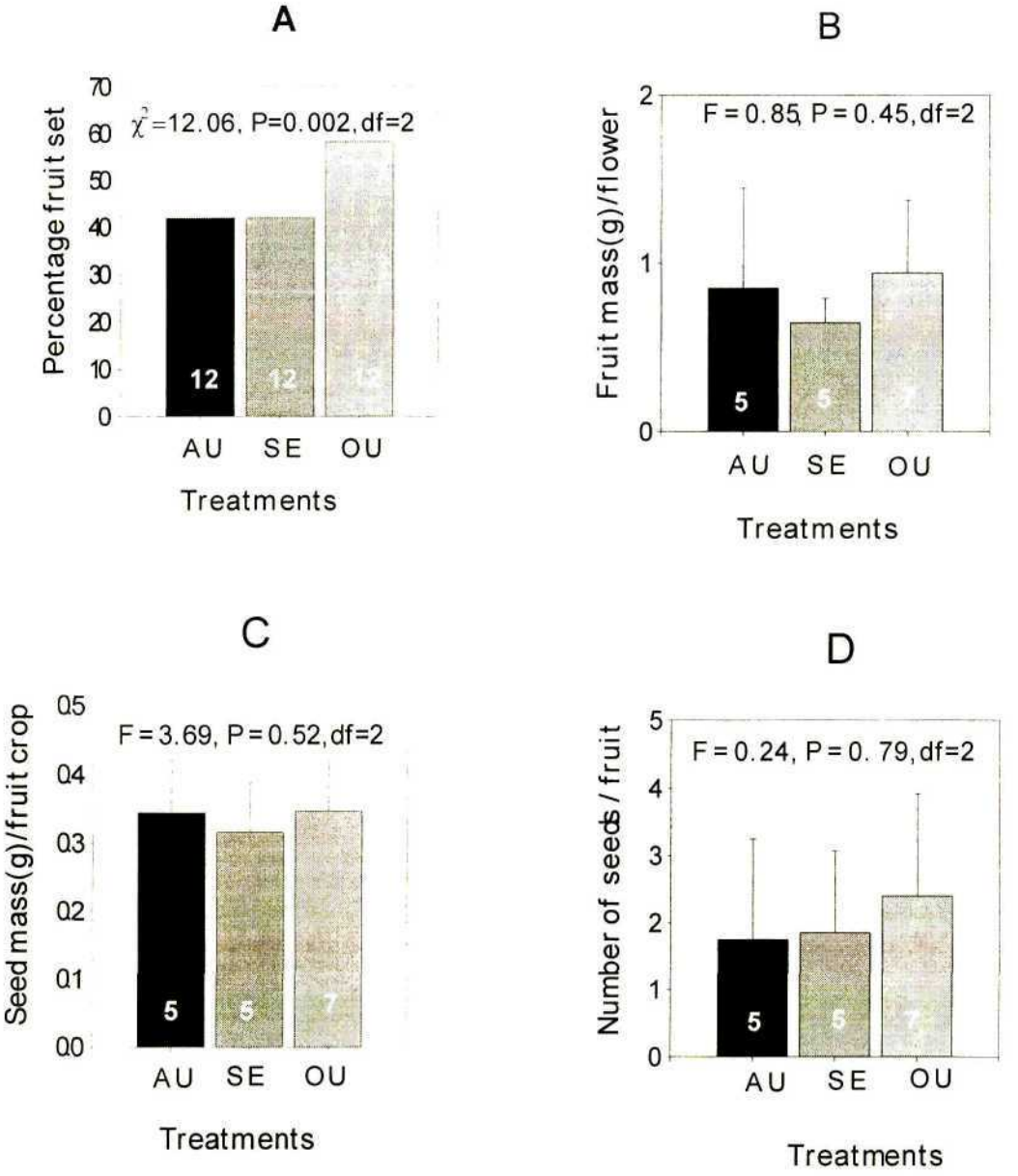

Figure 2.10. Breeding systems results for ***Ipomoea alba***. A. Percentage fruit set (n=number of flowers). B. Fruit mass (g)(n=number of fruits). C. Seed mass (g) (n=number of fruits). D. Number of seeds per fruit (n=number of fruits). AU= Autogamy, SE=Self-pollination and OU=Outcrossing. Error bars= standard deviation.

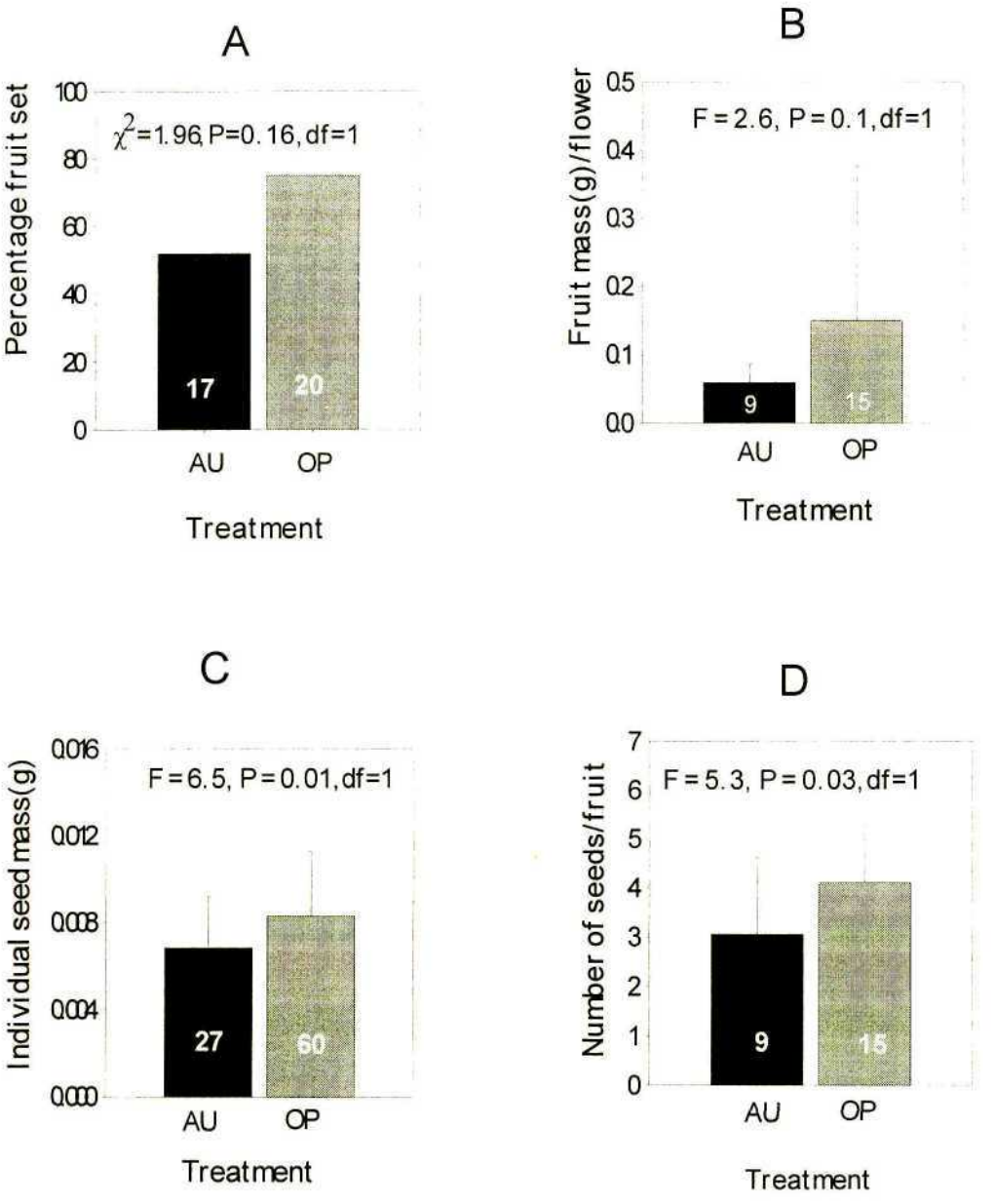

Figure 2.11. Autogamous breeding systems results for ***Acacia mearnsii***. A. Percentage fruit set (n=number of flowers). B. Fruit mass (g) (n=number of fruits). C. Seed mass (g) (n=number of seeds). D. Number of seeds per fruit (n=number of fruits). Error bars represent the standard deviation.

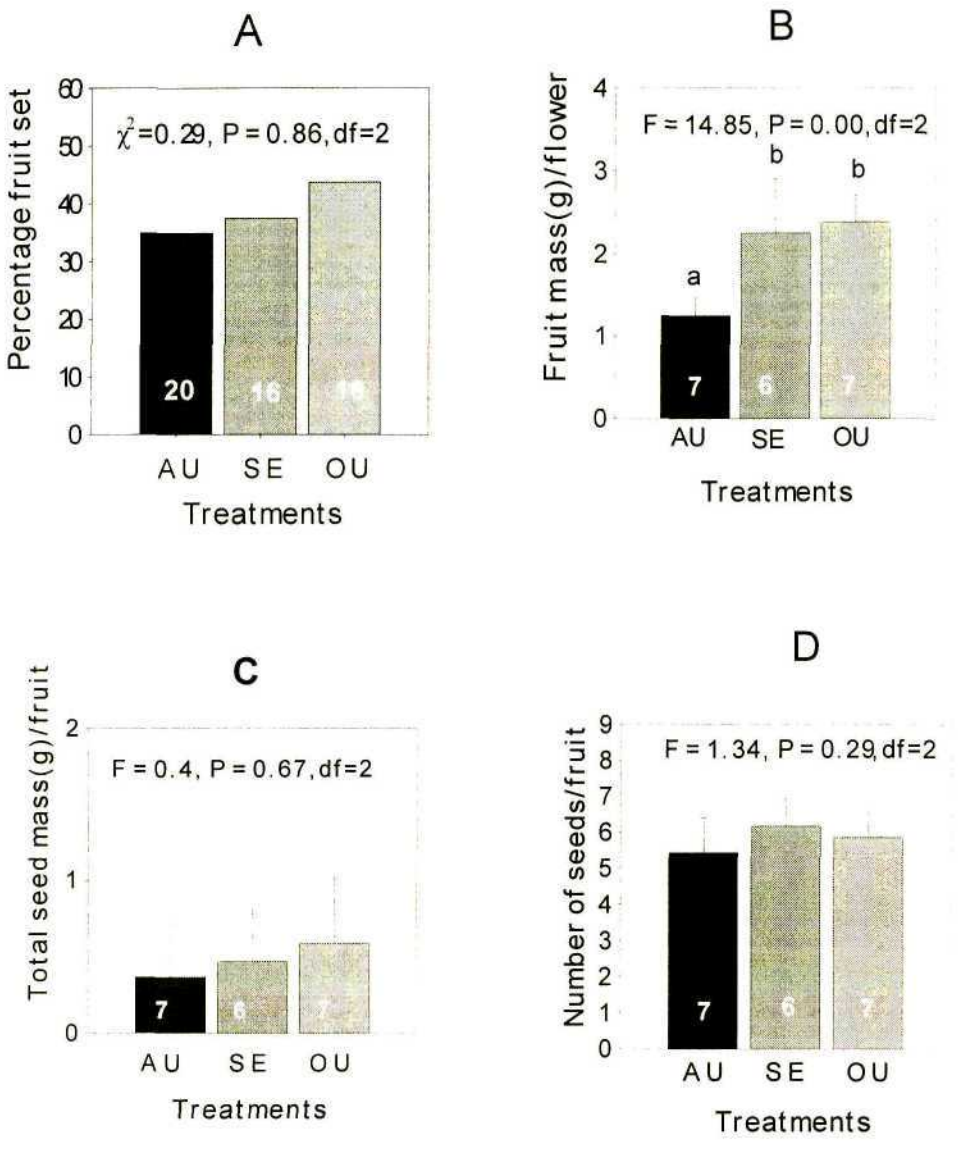

Figure 2.12. Breeding systems for ***Caesalpinia decapetala***. A. Percentage fruit set (n=number of flowers). B. Fruit mass (g)(n=number of fruits). C. Seed mass (g)(n=number of fruits). D. Number of seeds (number of fruits). AU=Autogamy, SE=Self-pollination and OU=Outcrossing, AU=Autogamy, SE=Self-pollination and OU=Outcrossing. Error bars=standard deviation.

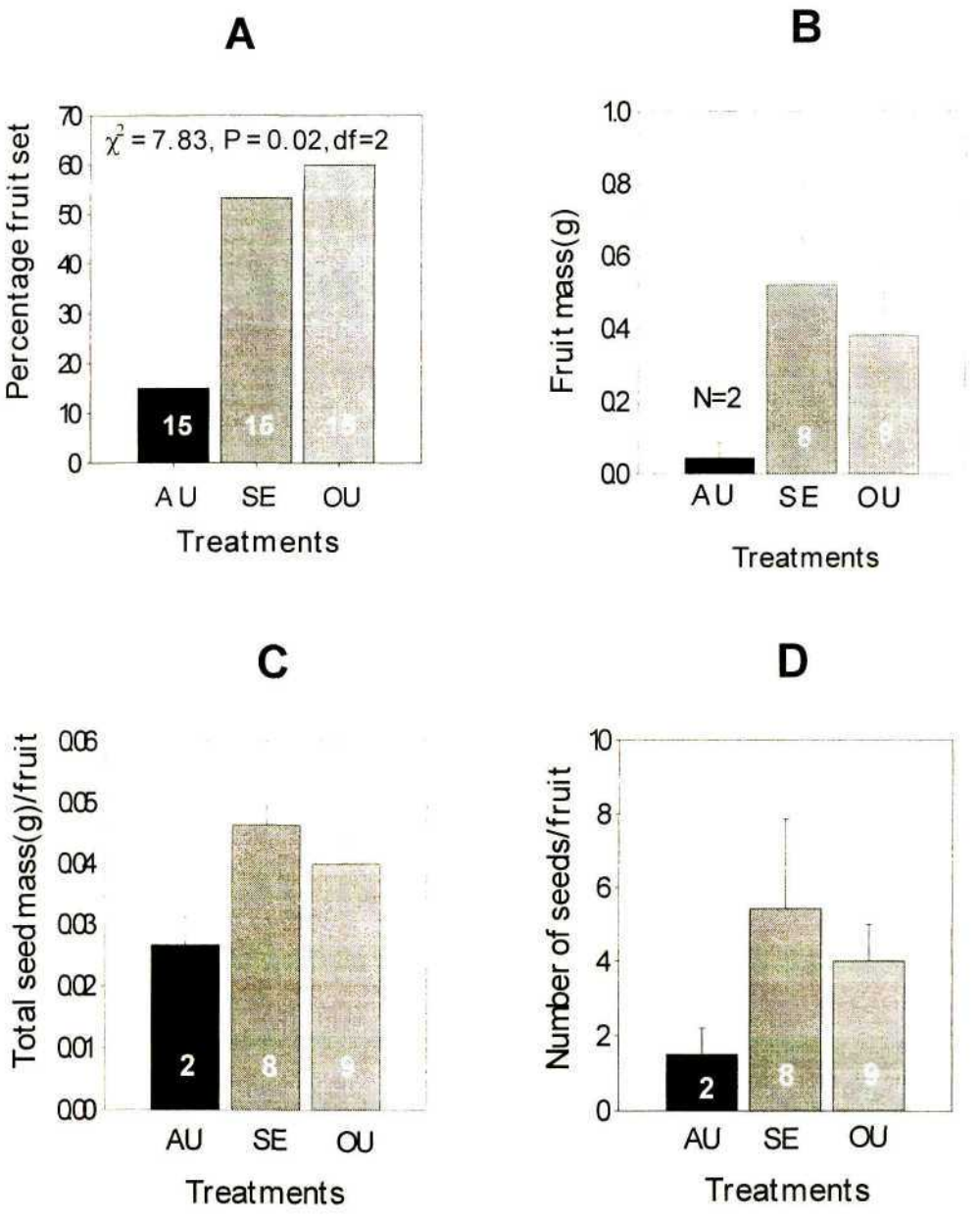

Figure 2.13. Breeding systems for ***Senna didymobotrya***. A. Percentage fruit set (n=number of flowers). B. Fruit mass (g) (n=number of fruits). C. Seed mass (g)(n=number of fruits). D. Number of seeds (n=number of fruits). AU=Autogamy, SE=Self-pollination and OU=Outcrossing. Bars with same symbols are not significantly different from each other. Error bars= standard deviation.

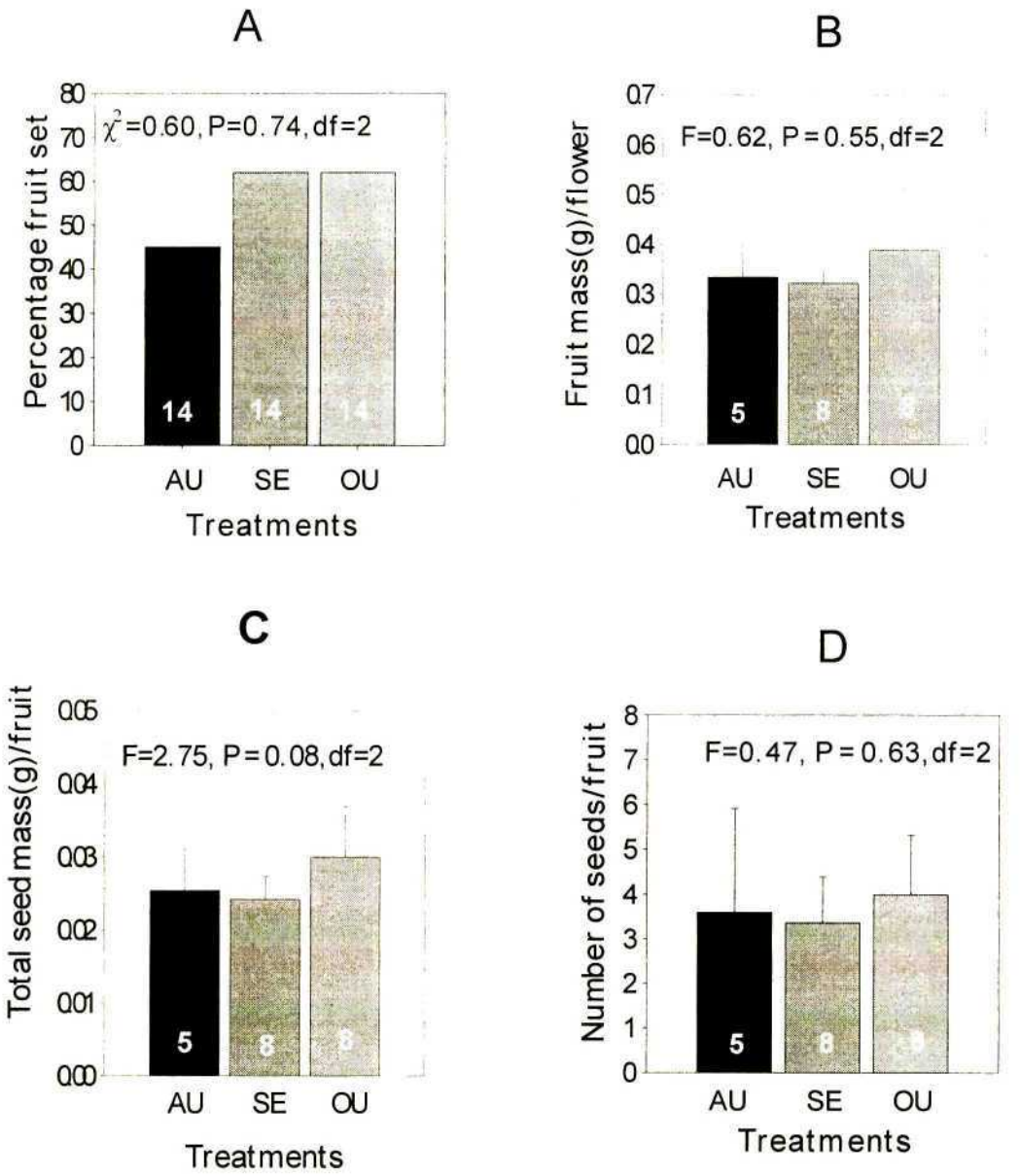

Figure 2.14. Breeding systems results for ***Sesbania punicea***. A. Percentage fruit set (n=number of flowers). B. Fruit mass (g) (n=number of fruits). C. Seed mass (g) (n=number of fruits). D. Number of seeds per fruit (n=number of fruits). AU=Autogamy, SE=Self-pollination and OU=Outcrossing. Error bars= standard deviation.

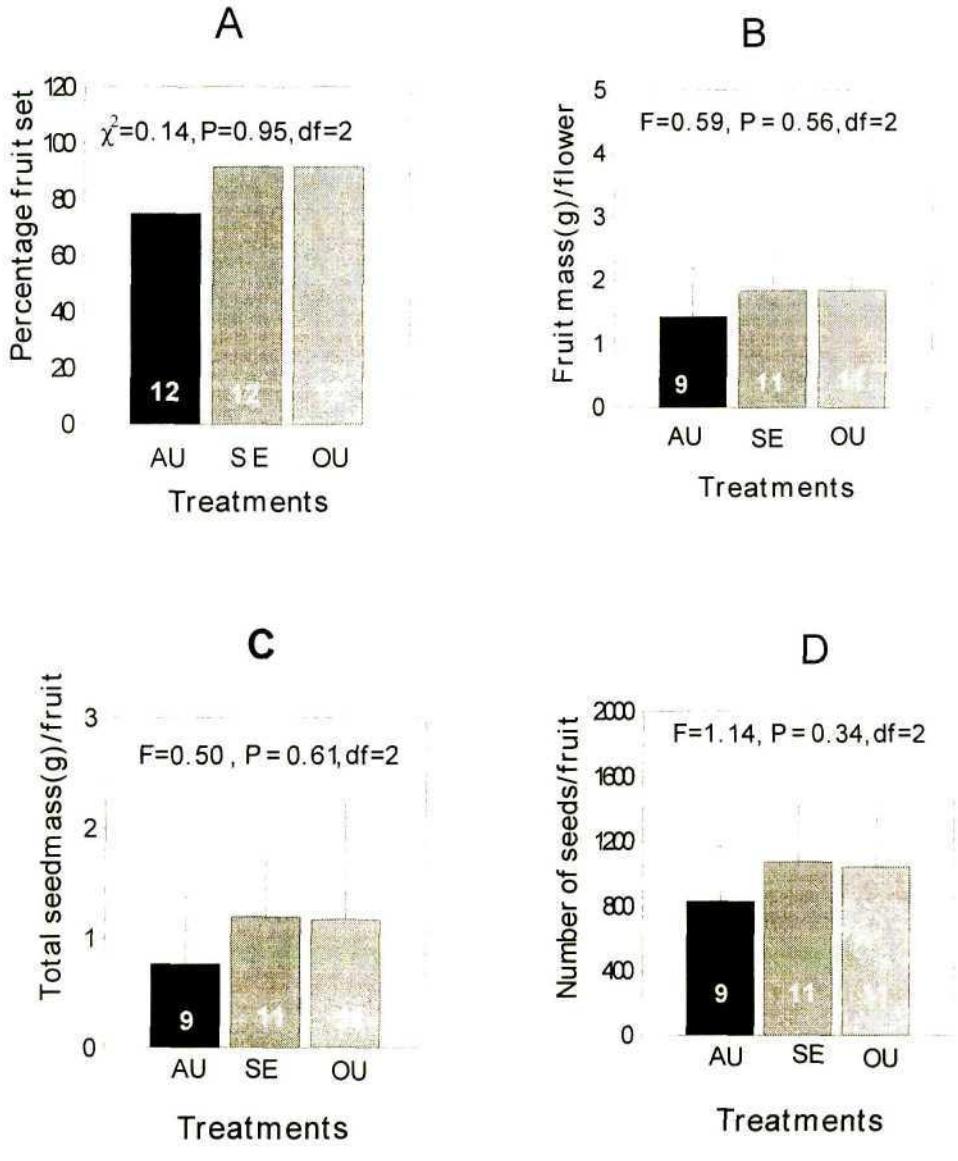

Figure 2.15. Breeding systems results for ***Lilium formosanum***. A. Percentage fruit set (n=number of flowers). B. Fruit mass (g) (n=number of fruits). C. Seed mass (g)(n=number of fruits). D. Number of seeds per fruit (n=number of fruits). AU=Autogamy, SE=Self-pollination and OU=Outcrossing. Bars with same symbols are not significantly different from each other. Error bars= standard deviation.

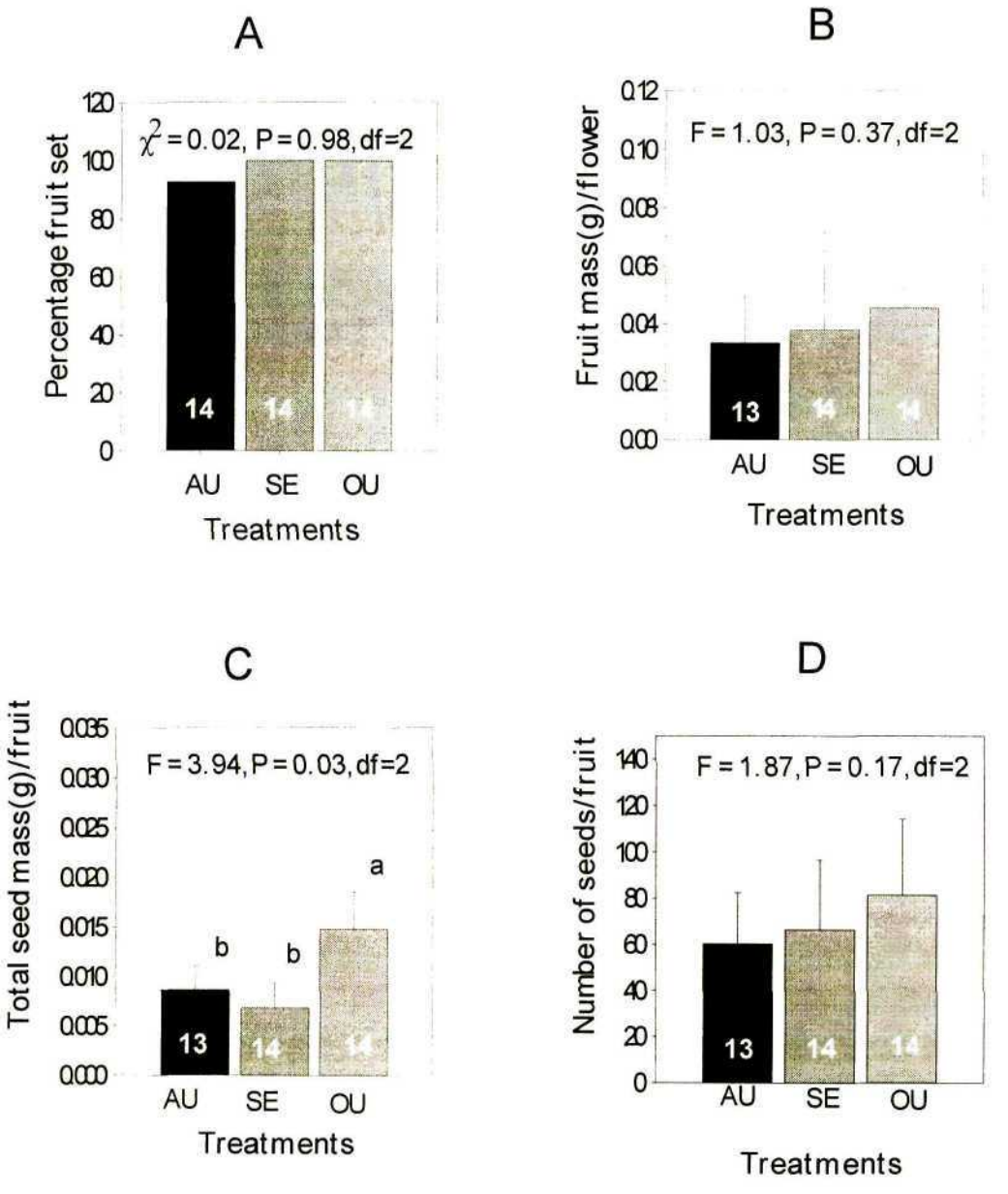

Figure 2.16. Breeding systems results for ***Eucalyptus camaldulensis***. A. Percentage fruit set (n=number of flowers). B. Fruit mass (g)(number of fruits). C. Seed mass (g)(n=number of fruits). D. Number of seeds per fruit (n=number of fruits). AU=Autogamy, SE=Self-pollination and OU=Outcrossing. Bars with the same symbols are not significantly different from each. Error bars= standard deviation.

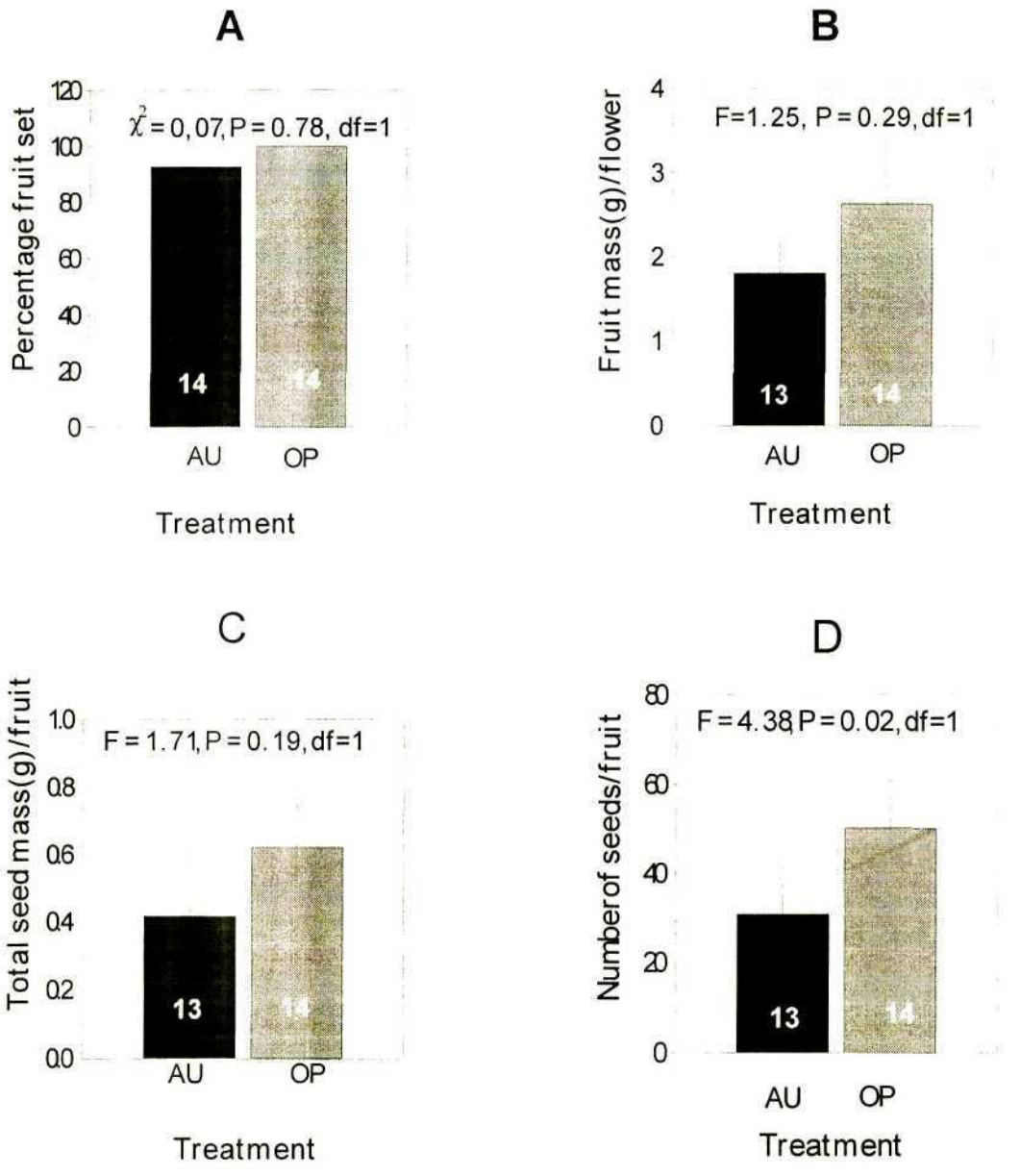

Figure 2.17. Breeding systems results for ***Psidium guajava***. A. Percentage fruit set (n=number of flowers). B. Fruit mass (g) (n=number of fruits). C. Seed mass (g) (n=number of fruits). D. Number of seeds per fruit (n=number of fruits). Error bars represent the standard deviation.

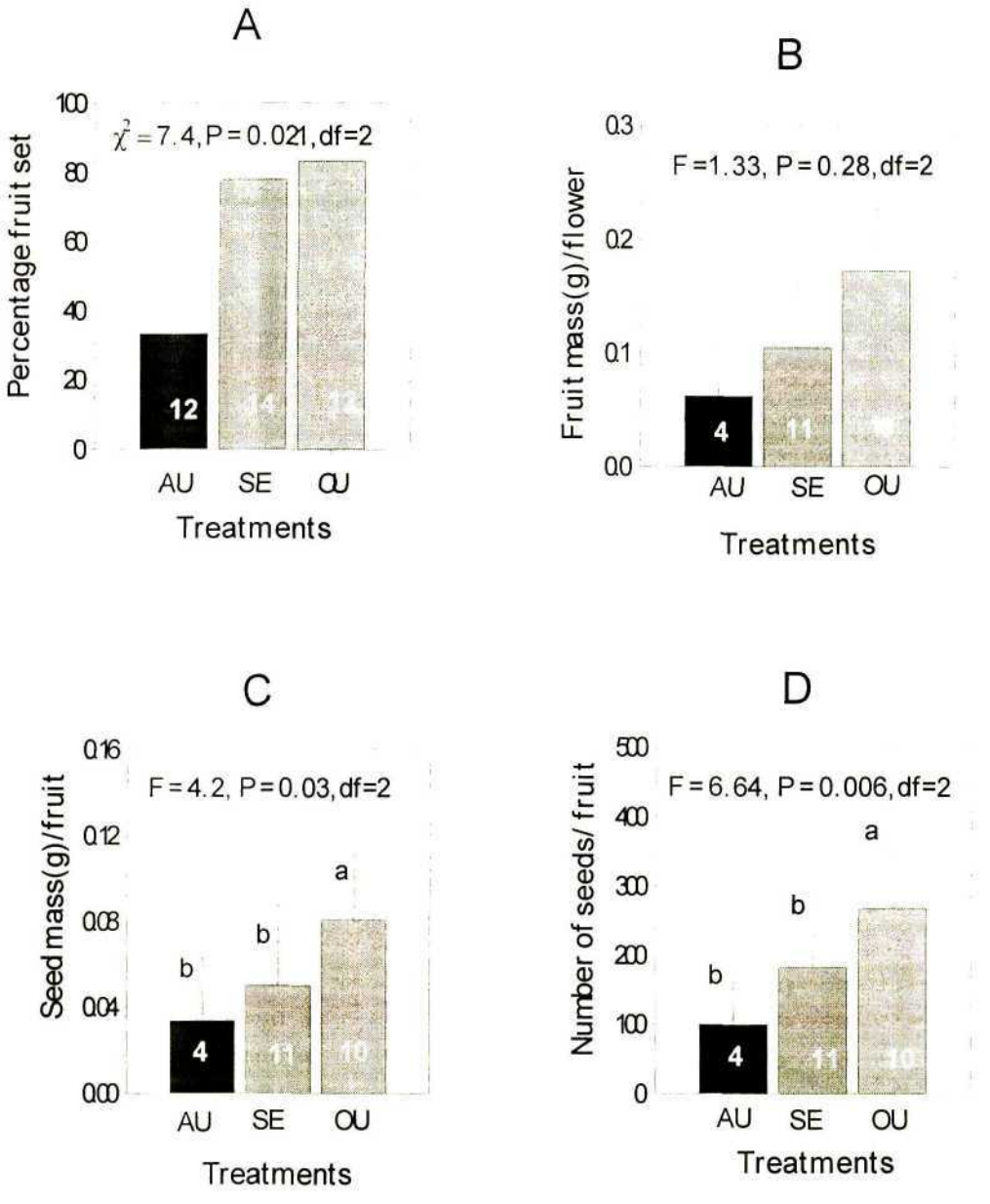

Figure 2.18. The breeding systems results for ***Oenothera biennis***. A. Percentage fruit set (n=number of flowers). B. Fruit mass (g)(n=number of fruits). C. Seed mass (g) (n=number of fruits). D. Number of seeds per fruit (n=number of fruits). Bars with the same symbols are not significant different to each other. Error bars represent the standard deviation.

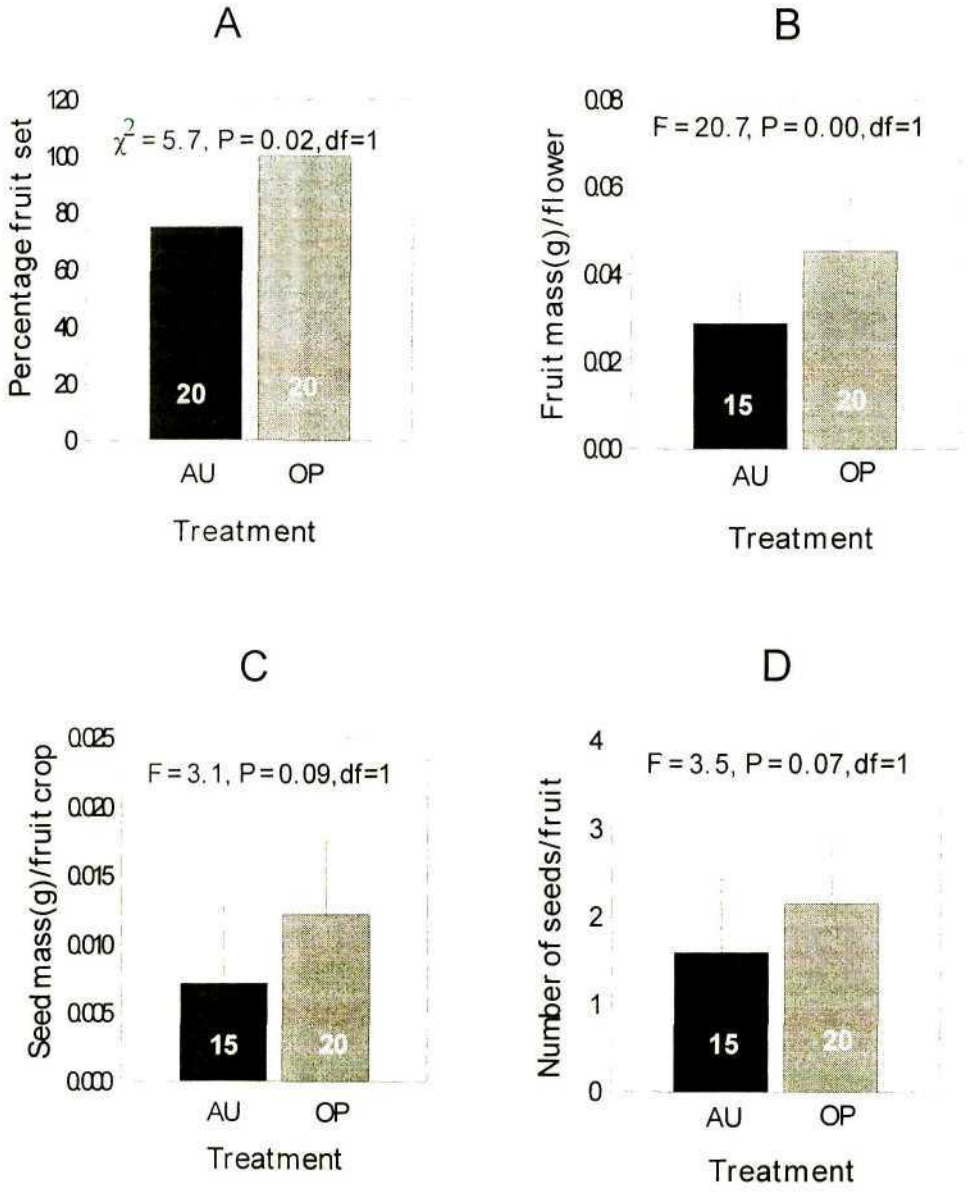

Figure 2.19. Breeding systems results for ***Cestrum aurantiacum***. A. Percentage fruit set (n=number of flowers). B. Fruit mass (g) (n=number of fruits). C. Seed mass (g) (n=number of fruits). D. Number of seeds per fruit (n=number of fruits). Error bars represent the standard deviation.

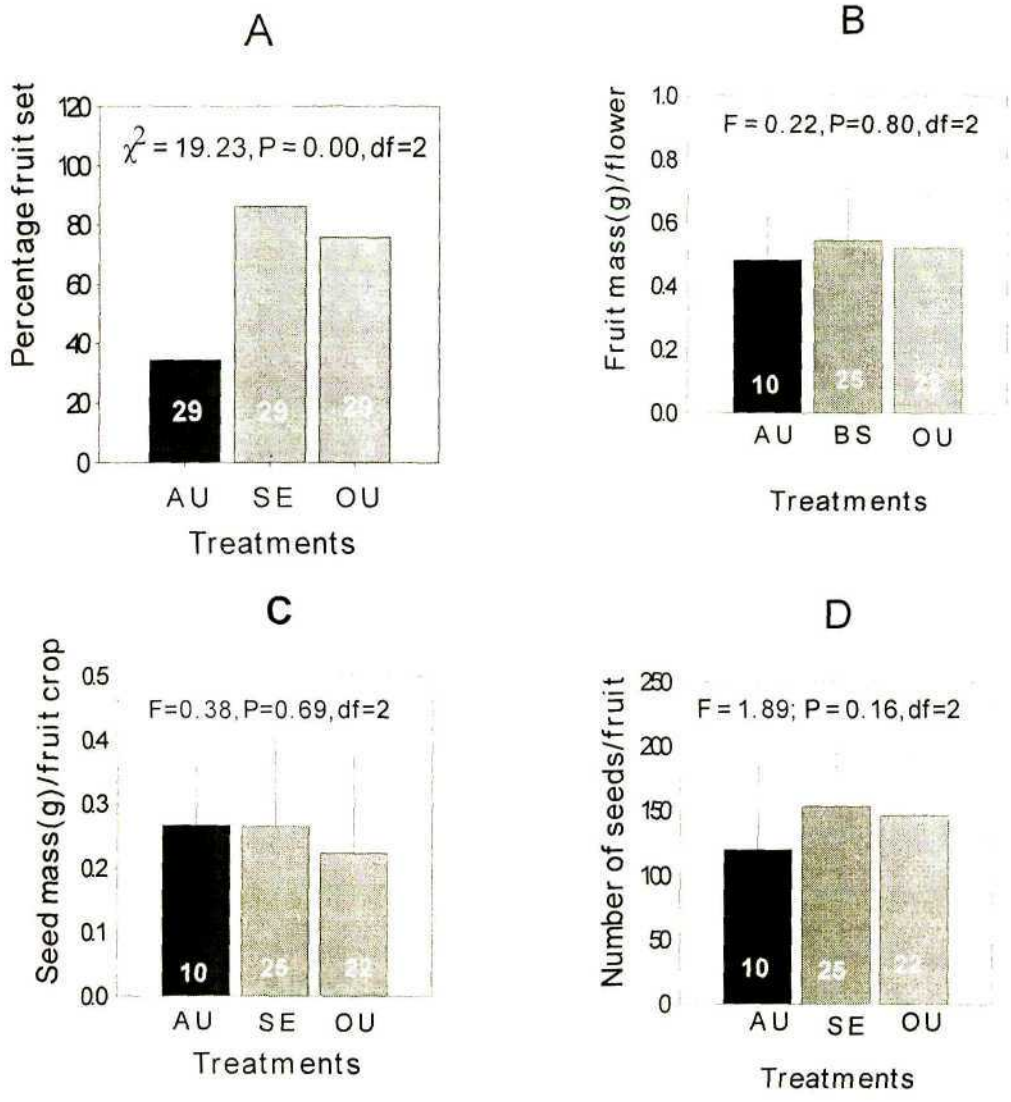

Figure 2.20. Breeding systems results for ***Solanum mauritianum***. A. Percentage fruit set (n=number of flowers). B. Fruit mass (g) (n=number of fruits). C. Seed mass (g) (n=number of fruits). D. Number of seeds per fruit (n=number of fruits). AU=Autogamy, SE= Self-pollination and OU=Outcrossing. Bars with same symbols are not significant different from each other. Error bars represent standard deviation.

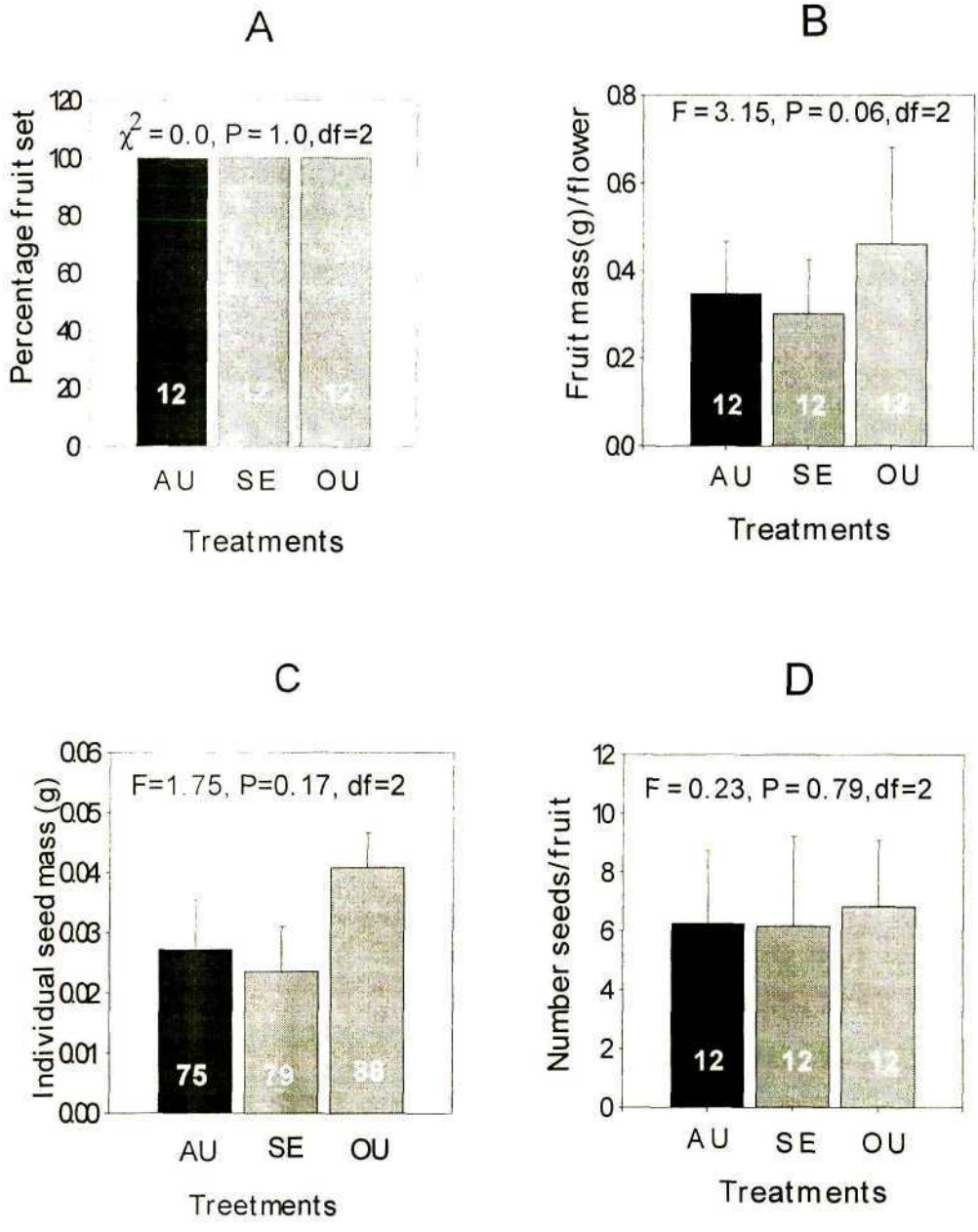

Figure 2.21. Breeding systems results for ***Hedychium gardnerianum***. A. Percentage fruit set (n=number of flowers). B. Fruit mass (g)(n=number of fruits). C. Individual seed mass (g)(n=number of seeds). D. Number of seeds per fruit (n=number of fruits). AU=Autogamy, SE=Self-pollination and OU=Outcrossing. Error bars= standard deviation.

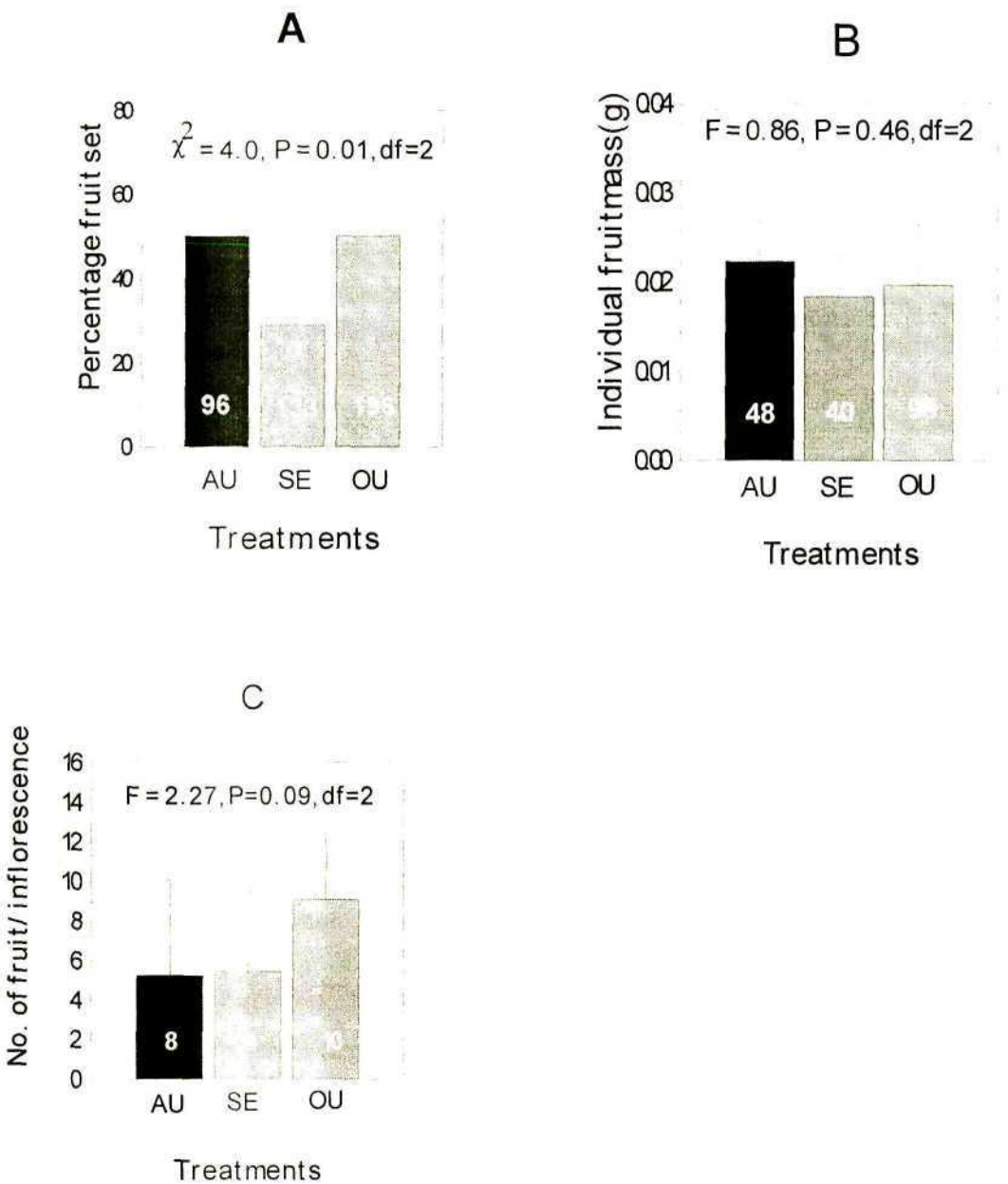

Figure 2.22. Breeding system results for ***Lantana camara***. A. Percentage fruit set(n=number of flowers). B. Individual fruit mass (g)(n=number of fruits). C. Number of fruits per inflorescence (n=number of inflorescence). AU=autogamy, SE=Self-pollination and 0U=Outcrossing. Error bars = standard deviation.

Discussion

This study demonstrates that there is a high level of autogamy and self-compatibility among the 18 alien invasive species sampled in South Africa. This result is consistent with Baker's (1955) prediction that colonizing species tend to have uniparental reproduction. Baker's (1955) concept of an ideal alien invasive plant was one that had a combination of short life span & self-fertility. Some of the characteristics consistent with Baker' ideal weed are found in herbaceous plants in this study (e.g. *Lilium formosanum*, *Hedychium flavum* and *Oenothera biennis*), but, importantly the tendency to uniparental reproduction is also found among woody invasive species.

Breeding system experiments have previously been undertaken on only a small proportion of the study species in their native habitats (Table 2.6). *Caesalpinia decapetala* was found to be self-compatible in this study (Fig. 2.12), but self-incompatibility has been reported in other non-invading species of Caesalpinia, such as *C. calycina, C. pluviosa* and *C. eriostachy* (Bawa, 1974; Bawa & Webb, 1984; Bullock, 1985; Lewis & Gibbs, 1999). *Lilium formosanum* was found to be autogamous (Fig. 2.15), but previous study in Taiwan suggests self-incompatibility in this species (Hi Song, 1996) (Table 2.6). *Lantana camara* was found to be autogamous (Fig. 2.22), while previous studies in India suggest self-compatibility in this species (Schemske, 1976; Mathur & Mohan Ram, 1986) (Table 2.6). *Oenothera biennis* was found to be self-compatible in this study (Fig. 2.18) and studies by Hall *et al.* 1988 on the biology of Canada weeds support this evidence. Similarly, *Senna didymobotrya* was found to be self-compatible in this study (Fig.2.13) and a study by Dulberger (1981) supports this result (Table 2.6).

The link between selfing and invasion of species confirms the previous literature on the reproductive success of colonizing species. A survey of the British flora by Price and Jain (1991) suggests that there is a strong association between colonization and self-compatibility among colonizing species. Self-fertility may have been strongly selected for in colonizing individuals, as many alien species are self-incompatible in their natural habitat but self-compatible in South Africa (for example: *O. biennis*

and *L. formosanum*). The selection for self-pollination is presumably a result of the advantages of uniparental reproduction for successful colonization ability.

It is interesting to consider whether self-fertility has evolved in colonizing species. Some of the species investigated are self-incompatible in their natural habitat; yet show a high level of selfing in South Africa (Table 2.6). Raven (1979) believed that some *Oenothera* species that immigrated to South America regained some degree of outcrossing in some populations through selection on flower morphology, and there is consequently a varying degree of outcrossing and self-pollination amongst populations. One may envisage the same situation in South Africa. *Oenothera biennis* showed limited autogamy in this study (Fig 2.18). The species might have colonized South Africa through autogamous breeding system and may have regained some degree of outcrossing.

According to Richards (1997), autogamy is an advantageous breeding system for alien invasive species because of the reduction of genetic variation and fixation of highly adapted genotypes. However, in colonizing species, fitness is highly reliant on reproductive success. It is evident that autogamous species such as *Canna indica*, *Hedychium gardnerianum*, *Lilium formosanum*, *Opuntia stricta* and *Psidium guajava* produce exceptionally high levels of fruit and seed set (Fig. 4.26).

Apomixis can be an advantage in species such as *Ageratina adenophora and Chromolaena odorata* that produce high numbers of seeds without fertilization. *Chromolaena odorata* is also able to perpetuate itself vegetatively as well as to produce high levels of seed set. This potentially makes it more of a serious invader than the other species.

Table 2.6: Comparison of breeding system results reported in this study with previous publications.

	Breeding systems		
Species	**Present study**	**Previous publication**	**Reference**
Acacia mearnsii	autogamy	self-compatible	Moffett (1956)
Lantana camara	autogamy	self-compatible	Mathur & Mohan Ram (1986)
Lilium formosanum	autogamous	self-incompatible	Hi Song (1996)
Oenothera biennis	autogamy	self-compatible	Hall *et al.* (1988)
Psidium guajava	autogamy	Autogamy	Purseglove (1968)
Senna didymobotrya	self-compatible	self-compatible	Dulberger (1981)
Solanum mauritiunum	self-compatible	self-compatible	Roe (1979)

Some species that are not invasive are nevertheless capable of high levels of seed set when self-pollinated. Breeding systems results show that *Cassia multijuga* (a non-invading species) is self-compatible and weakly autogamous (*T Rambuda*, unpublished data). The high level of reproductive success in the related invasive species of *Senna didymobotrya* can be attributed to high levels of visitation by *Xylocopa* bees (page 55).

Breeding systems and pollinator interactions

According to Kunin and Shmida (1997), natural selection favours self-compatibility in sparse populations. Sparse populations attract fewer and less loyal visitors than densely populated stands, which can result in a significant reduction in reproductive success (Kunin & Shmida, 1997).

Antonovics (1968) highlighted the advantages of self-fertility in introduced plants in the absence pollen vectors. However, he highlighted the fact that closely adjacent self-compatible plant species may develop homozygosity, which may be advantageous, because of fixation of adaptive characters. If all these factors are considered, self-fertility would be greatly advantageous for short-term

reproductive success, but outcrossing may be necessary for long-term persistence.

Most dioecious species are insect pollinated (Bawa, 1980). The absence of dioecious plants species in the world's weed flora, would seem to provide support for Baker's ideas. The problem with dioecy is that introduction requires two separate sexes, which will normally make it difficult for a dioecious plant to spread successfully in the absence of pollinator visitors. There are few dioecious species in introduced flora of South Australia, for example (Bawa, 1980). At the same time, a relatively high proportion of dioecious species in island floras provides a contradiction to this pattern (Bawa, 1980).

In New Zealand, a high percentage of dioecious species in the flora has led to suggestions that the island situation plays a role in the evolution of this breeding system. According to Bawa (1980), one answer was that the presence of outcrossing species on an island is an evolved situation and not a feature of recent colonization, like the one as discussed by Baker (1955). Li and Haufler (1999) found that genetic variability makes it more possible for plants to become established in an area for a long period of time. Thus, outcrossing species may be less likely to colonize new areas, but show greater persistence once colonization does occur.

One approach to the problem of whether self-fertility is advantageous for colonizing species is to compare colonizing species with non-colonizing species (Price & Jain, 1981). The approach was widely advocated by other workers (Baker & Stebbins, 1965; Deahler, 1998; Pannell & Barrett, 1998). This approach also permitted Baker (1965) to make a list of characteristics of an ideal weed. Such an approach would enable us to confirm whether there is a trend to self-pollination among alien invader species in South Africa. This was done by comparing breeding systems of alien plant invader species with a several survey of plants worldwide by Fryxell (1957)(Figure 5.32). This reveals a strong trend towards uniparental reproduction in alien species in South Africa compared with the world flora, thus lending strong support to Baker's (1955) rule.

Chapter 3

Plant pollination systems

Abstract

Plants that colonize new environments are likely to be exposed to a pollinator fauna that is different from that in their native habitat. Therefore, a generalist and flexible pollination system is a trait that would be expected in successful colonizers. Seventy one percent of the fourteen alien invasive species for which pollinators were obtained were pollinated by honeybees, which are super-generalists and ubiquitous in agricultural landscapes. However, most of the alien invasive species are autogamous and not totally dependent on honeybees for reproductive success. Some alien invasive species showed pre-adaptation to relatively specialized pollination systems, involving carpenter bees, hawkmoth and butterflies. Thus, alien invasive plants were not visited by a diversity of insects, but rather showed pre-adaptation to one or more pre-existing guilds in the new habitat.

Introduction

The degree of specialization in pollination systems may influence the chance of successful colonization of new environment by alien plant species. Species that are adapted for pollination by a wide variety of pollinators (a generalist pollination system) would be expected to be more successful than species that are pollinated by a specific type of pollinator (specialist pollination system) (Baker, 1955; Johnson & Steiner, 2000).

Not all insects that visit flowers are effective in removing and depositing pollen on conspecific stigmas. It is thus essential in any study of plant-pollinator interactions to determine the effectiveness of pollinators (Dieringer, 1992). Measures of pollination effectiveness include the number of compatible pollen grains deposited on the stigma or removed from anthers in a single visit (Motten, 1983; Snow & Roubik, 1987; Herrera, 1987; Waser & Price, 1990), or the number of seeds resulting from a single visit to a virgin flower (Beattie, 1972; Motten, 1983; Lindsey, 1984; Motten *et al.*

1981; Dieringer, 1992; Kearns & Inouye, 1993). In addition to single visit, the importance of different animals as pollinators is dependant on relative rates of visitation to plants (Johnson & Steiner, 2000).

It is sometimes difficult to measure overall effectiveness of insects as pollinators because some insects may be common visitors, yet ineffective as pollen carriers (Fishbein & Venable, 1996) e.g. pollen stored on the hindlegs of honeybees, does not necessarily come into contact with the stigma (Westerkamp, 1991). Preliminary observations had shown that alien invasive species are frequented by honeybees. Observations of pollinators and pollination efficiency experiments can determine the effectiveness of honeybees.

Mathur and Mohan Ram (1978) found that thrips play an important role in the pollination of *Lantana camara*. Thrips are notorious flower visitors and dwellers. Their piercing mouth parts enable them to pierce through pollen grains and suck the protoplast (Grinfel'd, 1959). Kevan (1983) concluded that because of this ability they are also able to extract nectar from the plant. In South Africa, the effectiveness of thrips on *Lantana camara* has not been tested.

The aim of this chapter is to study the degree of specialization versus generalization in the pollination systems of a sample of alien invasive plant species in South Africa. The hypothesis being tested is that generalist pollination systems are dominant amongst alien invasive species because generalist pollination systems may be more flexible.

Materials and Methods

Pollinators observations

Observations of pollinators were undertaken for 19 alien invasive species. Observations of diurnal insects on each species were carried out in a patch of flowers of 1m x 1m for a period of 5 hours per day for 10 days. Observations were made from 08h00 in the morning to13h00 in the afternoon.

Observations for nocturnal visitors was conducted from 18h00-10h00 in the evening for a period of five days. Nocturnal moth pollinators were captured using a light trap. The types and number of insect visits were recorded and voucher information is given in appendix. Insects were observed and caught at the time of probing for nectar or foraging for pollen, and killed with ethyl acetate in a killing jar. Pollen loads were removed from insects by rubbing fuschin jelly over the insect (Beattie, 1972). Fuschin jelly was melted onto a slide under a coverslip. A reference collection of pollen for each plant species was established by removing pollen from the anthers and mounting on a slide in fuschin jelly. The pollen from each insect was compared with pollen from the reference collection of pollen from plants each species. Pollen was counted under a compound microscope.

Visitation rate

The activity of pollinators at different times of the day were recorded. The behaviour of flower visitors was recorded using a portable dictaphone. Number of visits to each flower and timing of visits was established by playing back the tape in the laboratory.

Nectar measurements

In *Oenothera biennis* and *Caesalpinia decapetala*, nectar volumes were measured at different times of the day using micro pipettes. Sugar concentrations were estimated using a pocket refractometer.

Pollinator effectiveness

The effectiveness of pollinators was quantified in terms of their contribution to seed set in the field. Virgin flowers were exposed to a single visit by an insect and bagged to avoid any further visit. Fruit and seed set were compared to flowers that were not exposed to single visit and those exposed to open pollination. This technique has been widely applied in many agricultural field experiments as well as natural systems (Todd & McGregor, 1960; Free, 1970a; Kevan *et al.* 1990).

Effectiveness of thrips as pollinators of *Lantana camara* and *Solanum mauritianum*.

The effectiveness of thrips (in their wingless crawling stage) as pollinators was tested in *Lantana camara* and *Solanum mauritianum*. Plantex™ (a tacky substance which is applied onto the stem to prevent crawling insect from gaining access to the floral parts of the plant) was placed around 30 inflorescence stalks at the bud stage. To test if thrips play any role in the pollination systems of *L. camara* and *S. mauritianum*, another 30 inflorescences without Plantex™ were marked as controls. A significant increase in fruit set in flowers without Plantex™ would suggest that thrips have an effect on pollen deposition.

Effectiveness of honeybees as pollinators of *Solanum mauritianum*

The effectiveness of honeybees as pollinators was tested for flowers of *Solanum mauritianum*. To determine whether honeybees contribute to the fruit set of this otherwise autogamous species, thirty-one flower buds were bagged at the bud stage and virgin flowers were exposed to honeybee visits. After each visit, flowers were bagged again to avoid visits by other insects, and fruit and seed set were later determined.

Effectiveness of diurnal and nocturnal insects as pollinators of *Oenothera biennis*

Although flowers of *Oenothera biennis* show traits that suggest hawkmoth pollination, such as long tubular flowers and even emission of scent, they are visited by honeybees during the day. To establish the relative importance of hawkmoths versus honeybees as pollinators of *O. biennis*, seed set was compared in flowers exposed only during the night (nocturnal) and flowers exposed only during the day (diurnal). Two groups of 15 plants were bagged at the bud stage. Flowers were exposed by removing the bags and exposing the virgin flowers and then replacing the bag. Daytime exposure (DE) was from 08h00 to 18h00 and night exposure was from 18h00 to 05h00. Flowers were bagged again after every exposure. Fruit set, fruit weight, seed weight and number of seeds were later determined.

Results

Observations and behaviour of pollinators

Among visitors, bees were the most common visitors to alien invasive plants. Species that were visited regularly by honeybees are *Solanum mauritianum*, *Oenothera biennis*, *Caesalpinia decapetala*, *Opuntia stricta*, *Jacaranda mimosifolia*, *Eucalyptus camaldulensis*, *Psidium guajava*, *Hedychium gardnerianum* and *Senna didymobotrya* (Table 3.7). *Caesalpinia decapetala*, *Senna didymobotrya* and *Sesbania punicea* were visited by large *Xylocopa* bees (Table 3.7). *Lantana camara* was visited by a wide range of butterfly species, including *Acraea agloanice*, *Acraea cabira*, *Acraea natalica*, *Amauris echeria*, *Catopsilia florella*, *Coenyropsis natalii*, *Danaus chrysippus*, *Eurema brigitta*, *Papilio nireus*, *Papilio demodocus* and *Vanessa cardui* (Table 3.7). Voucher specimens of different pollinators for each plant species are stored in the taxonomy laboratory of the University of Natal, Pietermaritzburg.

Acacia mearnsii

This species was visited by honeybees which collected pollen. Little time was spent per flowerhead by honeybees (1.4s) (Table 3.7) and they visited few flower heads per inflorescence (Table 3.7). The fact that the species is autogamous (Fig. 2.11) suggests that pollination by honeybees was negligible and pollinators contributed very little to fruit set.

Caesalpinia decapetala

Large bees (*Xylocopa flavorufa*) were observed grasping the petals and forcing their heads into the flower to access nectar from *Caesalpinia decapetala*. While feeding, the bee's abdomen curls around the anthers and accumulates a large number of pollen grains (ca. 10000 grains) on its body termed buzz pollination (Table 3.7). Honeybees were also observed to visit *C. decapetala* frequently, but very little pollen was carried by these insects ($\bar{x}$ = 15.3 grains) as they seldom contact the anthers while feeding (Table 3.7). *Xylocopa flavorufa* spent a short time on each flower ($\bar{x}$ = 7.6s). Honeybees spent more time than *Xylocopa* per flower (ca. 10.2 s) and per inflorescence (ca. 14.5 s) (Table 3.7).

Jacaranda mimosifolia

Honeybees are frequent visitors to this species, but rarely contact the anthers or stigma when feeding on nectar from the base of the corolla. As a result pollen loads on honeybees were very small ($\bar{x}$ = 6.8 grains, Table 3.7). As the flowers open with the stigmas in close contact with the anthers, self-pollination is permissible, but breeding systems results were inconclusive (Chapter 2).

Hedychium gardnerianum

Hedychium gardnerianum was visited by honeybees but visits were not frequent (Table 3.7). Breeding system experiments showed 100 percent fruit set due to autogamy, thus bees are probably not important for fruiting success.

Eucalyptus camaldulensis

This species was visited by honeybees (*A. mellifera*) which carried substantial amounts of pollen ($\bar{x}$ = 5063.6 grains) (Table 3.7).

Oenothera biennis

Oenothera biennis was frequently visited by honeybees. Honeybees carried a large amount of pollen ($\bar{x}$= 425.0 grains) on their bodies (Table 3.7). The bees in most cases went straight for nectar at the entrance to the flower. Honeybees seldom come into contact with the stigma of *O. biennis*. The stigmas protrudes from the flower and therefore it is difficult for honeybees to come into contact with the stigma. Bee's visitation was prevalent in the morning from 07h00 to 09h30. Honeybees carried substantial amounts of pollen grains (Table 3.7). However, contact of pollinators with the stigma was not frequent.

Although the long floral tube of *O. biennis* suggests hawkmoth pollination, no hawkmoths were observed or captured over a period of four hours and four nights with a light trap.

Opuntia stricta

This species was regularly visited by honeybees. Honeybees collected pollen and also fed on nectar. Moderate pollen loads ($\bar{x}$ = 118.0) were present on the bees (Table 3.7).

Sesbania punicea.

An extensive amount of pollen (> 2000 grains) was carried by individuals of *Xylocopa flavorufa* caught on flowers of *Sesbania punicea.* Therefore, *X. flavorufa* potentially contributes substantially to pollination of this species (Table 3.7). The bee curls its body around the floral parts immediately after landing and makes a high frequency vibration with a high pitch sound (buzz pollination).

Solanum mauritianum

Honeybees were active visitors of this species from morning until late in the afternoon (from 9h00 to 15h00). Despite the poricidal anther structure being typically associated with buzz pollination, honeybees do not buzz, but curl around the floral parts and collect pollen on their legs and body parts ($\bar{x}$ = 2866.6 grains) (Table 3.7) (see Figure 1.3 B). Pollen is easily released from the poricidal anthers. By shaking anthers with your finger, one can easily see a cloud of pollen from the poricidal anthers. No other species of bees were observed visiting this species. The time spent per visit by honeybees suggest that honeybees spend a lot of time within the inflorescence ($\bar{x}$ = 85.3 seconds) and less time is spent per inter-plant movement ($\bar{x}$= 2.1 seconds)((Table 3.7).

Senna didymobotrya

This species is effectively buzz pollinated by large *Xylocopa flavorufa* bees(see Figure 1.3 C). An enormous amount of *S. didymobotrya* pollen was carried by these bees ($\bar{x}$ = 14700.0 grains) (Table 3.7). The bees curl their bodies around the poricidal anthers and display a high frequency vibration with a high pitched buzzing sound. Honeybees were also observed visiting but this species accumulated only a small amount of pollen on their body ($\bar{x}$ = 78.3 grains)(Table 3.7). In addition, honeybees rarely made contact with the stigma.

Ipomoea alba

This species was visited at dusk by the hawkmoth *Agrius convolvuli*, which has a proboscis length of 117mm. Pollen loads on the hawkmoths were surprisingly small ($\bar{x}$ = 29.2 grains), despite the contact of the hawkmoths with the anthers (Table 3.7). The flower has a long floral tube length (118mm) which is closely matched to the tongue of the hawkmoths.

Lantana camara

Lantana camara was visited by a wide range of butterflies (Table 3.7) and also thrips. Butterflies probe flowers that are in their yellow stage and typically avoid flowers in their red stage because these flowers are already depleted of nectar. Close-up examination of flowers revealed numerous wingless thrips crawling inside the corolla (personal observation). It was not possible to expose virgin flowers to each butterfly visit. However, it was possible to measure the behaviour of different butterflies and their relative pollen loads (Table 3.7).

Cestrum aurantiacum

Cestrum aurantiacum was visited by butterflies (*Danaus chrysippus* and *Acraea cabira*), but the extent to which each species is effective as a pollinator was not determined.

Chromolaena odorata and *Ageratina adenophora*

No insect visits were observed to the flowers of *Chromolaena odorata* and *Ageratina adenophora* Reproduction is apomictic in both of these species.

Psidium guajava

Psidium guajava was visited by honeybees. Honeybees curl around the feathered anthers with the stigma in the middle of the flower. This species showed a high level fruit set through autogamy (Chapter 2). Pollen loads carried by honeybees were not determined and it was not known whether *A. mellifera* contributes to fruit set in this species.

Table 3.7: Pollinators recorded for alien plant species, and their proboscis length, pollen load and behavioral activity. The locality data for voucher specimens is given in Appendix 1.

Plant species	Pollinators (Family name)	N	Proboscis Length $\bar{x} \pm SD$	No. of pollen grains $\bar{x} \pm SD$	Time (s) visit /flower $\bar{x} \pm SD$	No. visits / inflorescence $\bar{x} \pm SD$
Acacia mearnsii	*Apis mellifera* (Apidae)	10	3.9 ±0.2	93.1 ± 83.4	1.4 ± 0.6	1.9 ± 1.0
Caesalpina decapetala	*Apis mellifera* (Apidae)	23	4.4 ± 0.5	15.3 ± 29.1	Ca. 10.2	Ca. 14.5
	Xylocopa flavorufa (Anthophoridae)	10	9.6 ± 0.9	Ca. 10 000.0	7.6 ± 5.4	2.5 ± 1.2
Jacaranda mimosifolia	*Apis mellifera* (Apidae)	20	4.0 ± 0.0	6.8 ± 16.1	8.4 ± 4.4	1.5 ± 0.6
Hedychium gardnerianum	*Apis mellifera (Apidae)*	10	4.0 ± 0.2	40.0 ± 9.8	?	?
Eucalyptus camaldulensis	*Apis mellifera* (Apidae)	11	4.4 ± 0.5	5063.6 ± 5857.1	5.9 ± 3.1	2.6 ± 1.0
Oenothera biennis	*Apis mellifera* (Apidae)	10	4.0 ± 0.2	425.0 ± 685.9	?	?
Opuntia stricta	*Apis mellifera* (Apidae)	10	3.6 ± 0.5	118.0 ± 43.6	?	?
Sesbania punicea	*Apis mellifera* (Apidae)	10	4.5 ± 0.2	76.2 ± 58.0	1.7 ± 1.1	4.1 ± 1.4
	Xylocopa flavorufa (Anthophoridae	10	9.2 ± 1.6	2008.7 ± 29.1	5.9 ± 3.1	?
Solanum mauritianum	*Apis mellifera* (Apidae)	10	4.2 ± 0.3	2866.6 ± 2642.1	85.3 ± 49.7	2.1 ± 1.1
Senna didymobotrya	*Apis mellifera (Apis mellifera)*	10	4.3 ± 0.4	78.3 ± 42.3	?	?
	Xylocopa flavorufa (Anthophoridae)	20	9.6 ± 1.7	14700.0 ± 10523.7	1.4 ± 0.6	?
Ipomoea alba	*Agrius convolvuli* (Sphingidae)	7	117.0 ± 0.0	29.2 ± 35.6	?	?
Lantana camara	*Acraea agloanice* (Acraeidae)	3	8.0 ± 0.2	40.0± 51.9	4.3 ±3.3	4.9 ± 1.6
	Acraea cabira (Acraeidae)	3	20.0 ± 0.3	30.0 ± 9.3	1.6 ± 0.6	1.7 ± 0.26
	Acraea natalica (Acraeidae)	10	10.0 ± 0.6	2.0 ±1.3	3.3 ±0.9	3.6 ± 1.7
	Amauris echeria (Nymphalidae)	8	10.5 ± 0.5	11.0 ± 9.1	1.7 ± 1.2	1.8 ± 27. 4
	Catopsilia florella (Pieridae)	4	8.0 ± 0.4	0.0	1.5 ± 1.5	2.7 ± 1.2
	Coenyropsis natalii (Satyridae)	10	10.0 ±0.5	37.0 ± 13.3	4.0 ± 0.9	1.0 ± 0.3
	Danaus chrysippus (Nymphalidae)	4	8.0 ± 0.2	0.0	1.0 ±2.3	1.5 ± 1.3
	Eurema brigitta (Pieridae)	5	?	?	1.4 ±0.6	1.6 ± 1.1
	Papilio nireus (Papilionidae)	5	10.2 ± 3.2	0.0	1.7 ± 0.7	2.4 ± 1.1
	Papilio demodocus (Papilionidae)	3	8.0 ± 0.2	9.0 ± 1.7	4.0 ± 4.2	4.5 ± 0.4
	Vanessa cardui (Nymphalidae)	5	10.0 ± 0.3	7.0 ± 0.2	4.0 ± 0.3	4.7 ± 0.7

Pollinator effectiveness

Effectiveness of thrips as pollinators of Lantana camara and Solanum mauritianum

Flowers of *Lantana camara* open more or less synchronously on an inflorescence. Thrips were observed in abundance, moving from one flower to another flower within the inflorescence. There was a significant difference in fruit set in flowers exposed to crawling insects (42.1%) compared to those from which crawling insects were excluded by Plantex™ (23.1%) (P = 0.02) (Fig 3.23 A). There was no significant difference in fruit set in inflorescences of *Solanum mauritianum* covered with Plantex™ (66.6 %) compared to inflorescences without Plantex™ (70.8 %) (P = 0.56) (Fig 3. 23 B).

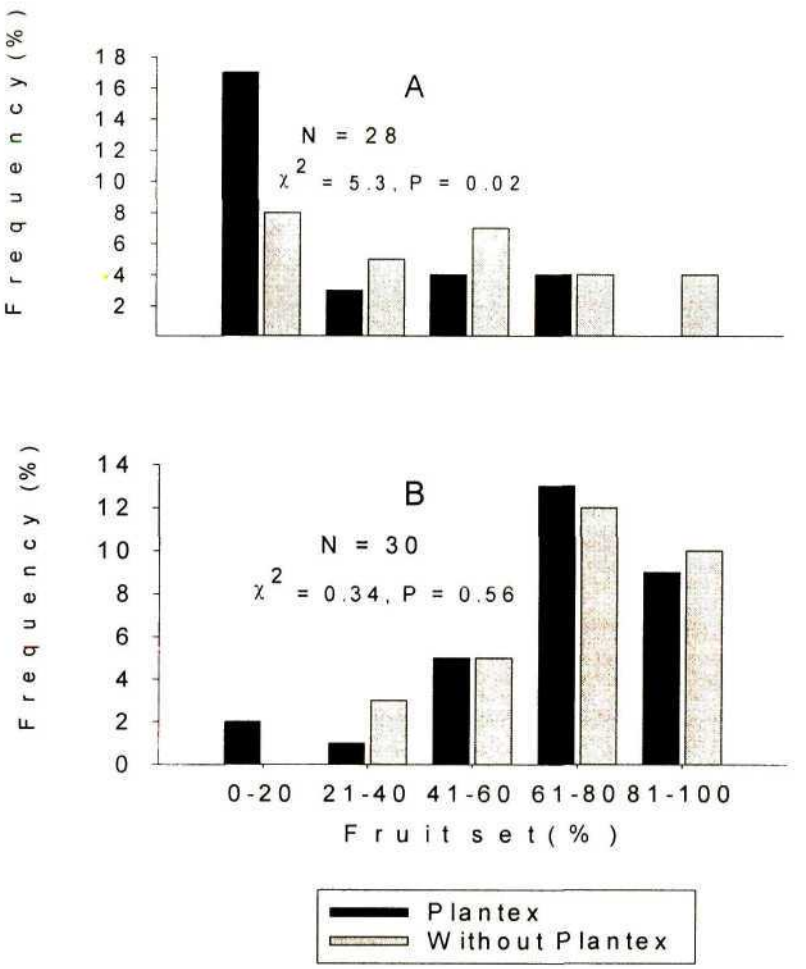

Figure. 3.23. Results of an experiment to determine the importance of crawling insects on *Lantana camara* (A) and *Solanum mauritianum* (B). Inflorescences with Plantex™ were not exposed to thrips and inflorescence without Plantex™ acted as controls exposed to thrips. In graph A & B the sample size refers to the number of flowers with Plantex™ and control (without Plantex™) treatments.

Effectiveness of honeybees as pollinators of Solanum mauritianum

The activity of honeybees was observed during the April/May period for one year. Flowers of *S. mauritianum* exposed to single visits by honeybees did not set significant higher levels of fruit set as compared to bagged flowers (Fig 3. 24 A). Fruit mass was significantly higher in open exposed flowers than bagged flowers (Fig 3.24 B). There was a significant difference in seed mass for flowers exposed to honeybees visits as compared to bagged flowers (Fig 3.24 C). The number of seeds per fruit was significantly higher in exposed flowers than in bagged flowers (Fig 3.24 D). These results indicate that honeybees contribute to seed set in *S. mauritianum*.

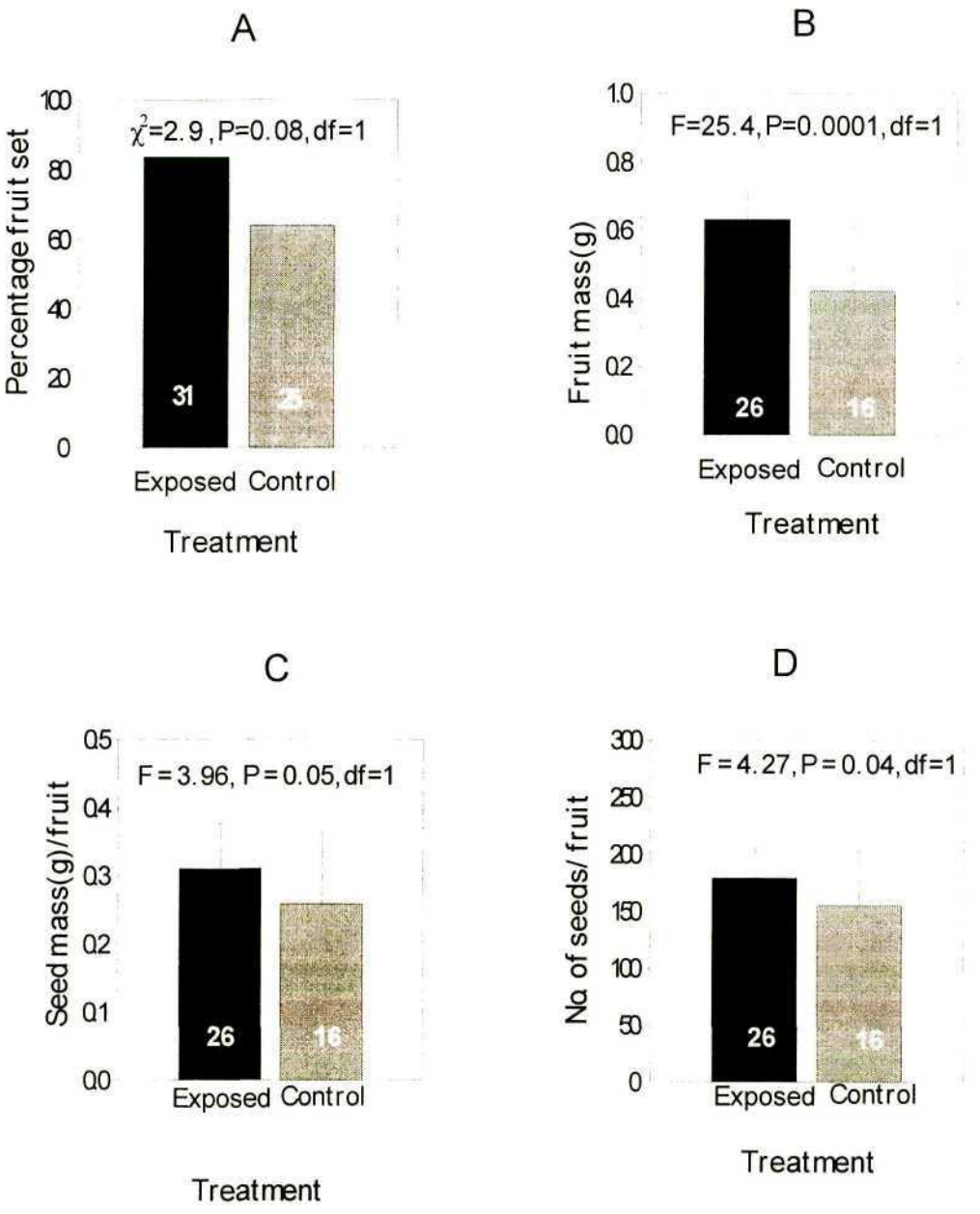

Figure 3.24. Effectiveness of honeybees *Apis mellifera* as pollinators of *Solanum mauritianum*. A. Percentage fruit set (n=number of treated flowers). B. Fruit mass (g)(n=number of fruits). C. Seed mass per fruit (g)(n=number of fruits). D. number of seeds per fruit (n=number of fruits). Error bars represent standard deviation.

Diurnal and nocturnal pollination in Oenothera biennis

There was a significant difference in fruit set between the autogamy treatment and flowers exposed to nocturnal and diurnal visitors (Fig 3.25 A). There was no significant difference in fruit mass or seed mass between a diurnal visit, nocturnal visit and autogamy treatments (Fig 3.25 B & C). Seed number was significantly lower in the autogamy treatment than in nocturnal and diurnal treatments (Fig 3.26 D).

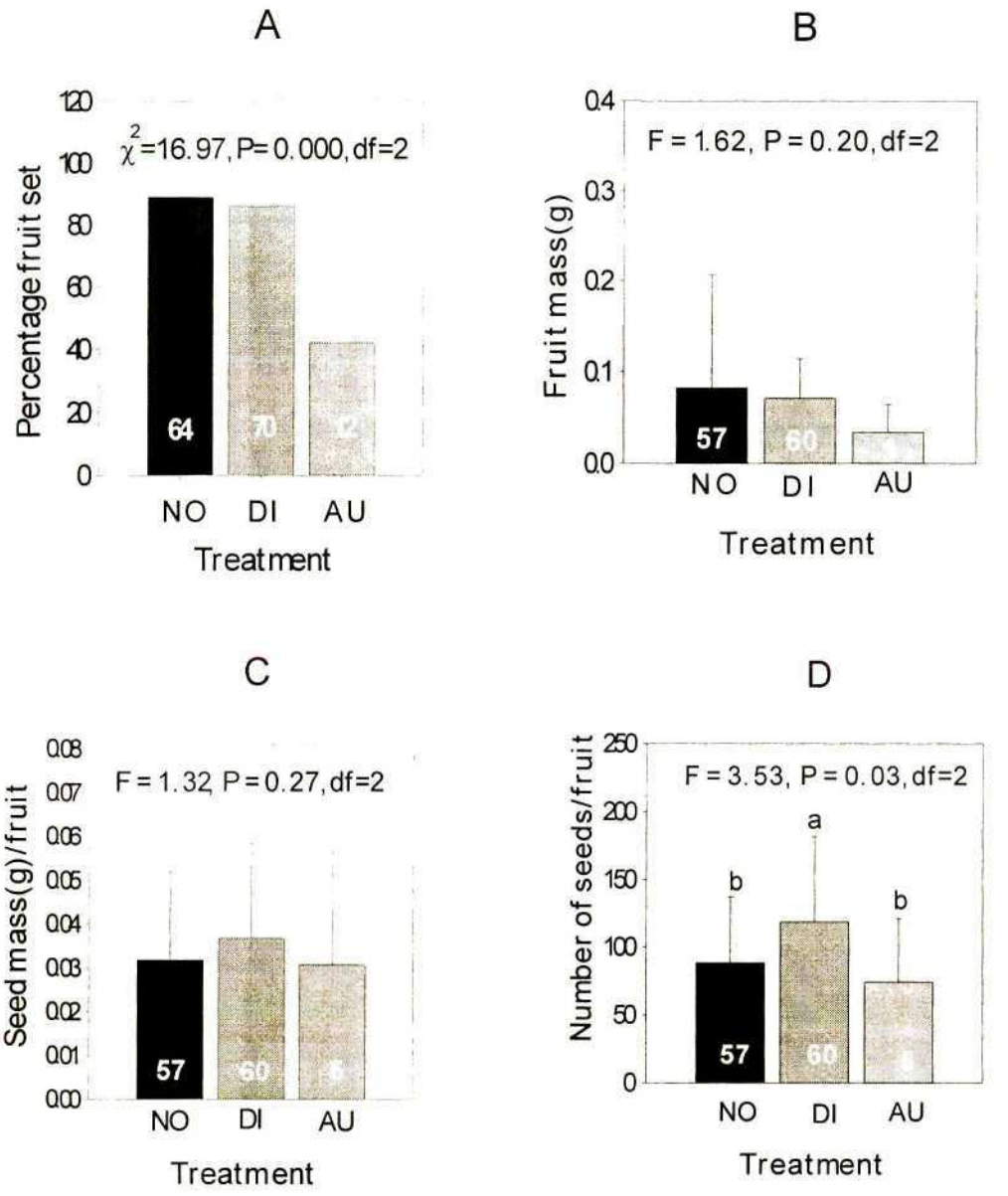

Figure 3.25. Reproduction success of flowers of *Oenothera biennis* exposed to nocturnal or diurnal visitor or neither. A. Percentage fruit set (n=number of flowers). B. Fruit mass (g)(n=number of fruits). C. Seed mass (n=number of fruits). D. Number of seeds per fruit (n=number of fruits). NO = nocturnal visit, DI = diurnal visit and AU = autogamy. Error bars on all graphs represent standard deviation.

Table 3.8. Pollen loads on honeybees (*A. mellifera*) from three different sites (Fig. 1.1). Site A = Ferncliffe, F = Sweetwaters, N = Hayfields and O = Howick (not in the map in Fig 1.1). Numbers inside the brackets represent the number of insects from which pollen loads were counted.

	Pollen count on *Apis mellifera*			
Species	Site A	Site F	Site N	Site O
Acacia mearnsii	93.14 ± 83.4(10)	260.0 ± 17.5 (10)		470.5 ± 77.2 (15)
Caesalpinia decapetala	1554.0 ± 158.9 (20)	5500.0 ± 143.2 (15)	15.26 ± 29.1(20)	
Solanum mauritianum	2866.6 ± 642.1 (15)	2160.0 ± 167.7(20)	655.0 ± 56.7 (15)	

Are honeybees consistent pollinators?

The pollen loads of honeybees (*A. mellifera*) from three sites in KwaZulu Natal are presented in table 3.8. These data demonstrate that honeybees are consistent pollinators of these three alien invasive plant species in this region and may be associated with more alien invasive plant species. Honeybees also visit many garden plants that are not indigenous (Personal observation).

Table 3.9. Nectar measurements for *Oenothera biennis*. Data is represented as $\bar{x}$ ± SD (n). N=number of flowers.

	Time		
Variables	07h30-10h30	11h00-14h00	14h30-18h00
Nectar volumes (µl)	5.8 ± 0.71 (30)	4.68 ± 0.63 (18)	5.2 ± 1.19 (30)
Sugar concentration (%)	17.4 ± 3.04 (30)	18.0 ± 7.01 (18)	19.7 ± 3.09 (30)
Number of honeybees	8	0	0

Table 3.10. Nectar measurements for *Caesalpinia decapetala*. Data is represented as $\bar{x} \pm$ SD (n). N=number of flowers.

	Time		
Variables	10h30-12h30	13h00-15h00	15h30-17h00
Nectar volumes (µl)	1.0 ± 0.57 (10)	0.58 ± 0.25 (9)	0.9 ± 0.43 (11)
Sugar concentration (%)	8.0 ± 2.5 (10)	5.7 ± 3.0 (9)	8.81 ± 3.0 (11)
Number of observed honeybees	76	14	0
Number of observed *Xylocopa* bees	8	3	0

Nectar production in Oenothera biennis

In *Oenothera biennis*, a substantial amount of nectar was present as a standing crop throughout the day and towards the evening (from 18h00 to 19h30)(Table 3.9). There was no significant difference in the volume of nectar produced in the morning, afternoon or towards the evening ($F = 1.18$, $df = 2$ and $P = 0.33$) (Table 3.9). There was no significant difference in sugar concentration between the morning and throughout the day ($F = 0.67$, $df = 2$ and $P = 0.54$) (Table 3.9).

Nectar production in Caesalpinia decapetala

There was no significant difference in nectar volume between the morning, middle of the day, or afternoon ($F = 2.25$, $df = 2$ and $P = 0.13$) (Table 3.10). There was also no significant difference in sugar concentration throughout the day ($F = 2.84$, $df = 2$ and $P = 0.08$) (Table 3.10). Honeybees predominatly extracted nectar from flowers in the morning and in the middle of the day (Table 3.10). *Xylocopa* bees were observed to extract both nectar and pollen. Their activity decreases during the day from 13h00 (Table 3.10).

Discussion

The extent that pollinators contribute to reproductive success of alien invasive species in KwaZulu Natal may be small. Alien invader species were visited by very few insect taxa and tend to have autogamous breeding systems. These findings confirm Baker's (1955) proposition that a generalized pollination system is common amongst colonizing species.

Plants are often pollinated by two or more insect orders despite having traits typical of being pollinated by a particular insect (Paton, 1993). Floral traits may not necessarily allow accurate predictions to be made about pollinators. For example, flowers of *Oenothera biennis* conform to the hawkmoth pollination syndrome i.e. long-tubed, pale, sweetly scented flowers which open at dusk and wither after dawn (Wyatt, 1983; Knudsen & Tollsten, 1993; Miyake & Yohara, 1998). However, in its country of origin, *O. biennis* is pollinated by hawkmoths as well as bees (Hall *et al.* 1988).

In *Oenothera biennis*, the contributions of nocturnal and diurnal visitors were about equal (Fig. 3.26). Recent observations by S.D. Johnson (personal communication) show that in addition to honeybees, both hawkmoths and noctuid moths visit the flowers and carry pollen loads of *O. biennis* in the KwaZulu-Natal midlands. Diurnal visitors contributed more to high fruit set than nocturnal visitors in a study of the contributions of diurnal and nocturnal insects to the pollination of common milkweed (*Asclepias syriaca* L.:Asclepiadaceae)(Morse & Fritz, 1983). Bertin and Wilson (1980), in their study of effectiveness of diurnal and nocturnal pollination of two milkweeds, found that nocturnal pollinators were able to insert and remove few pollinia compared to diurnal pollinators, but produced the same number of fruits. In a study of honeybee visits to *Oenothera* spp., *A. mellifera* did most of the morning visits to *O. deltoides* and *O. californica* (Gregory, 1964) between 7:30 and 9:15 (Gregory, 1964). However, flowers of *Oenothera* species were also regularly visited by various species of bees and night flying moths in the USA (Hoff, 1961).

The knowledge of pollination by insects that result in high fruit set is also important for crop production. Fruit set and marketability of cantaloups (*Cucumis melo*) in Yuma, Arizona, were

correlated with honeybee visits (McGregor *et al.* 1965). A study by McGregor and Todd (1952) showed that more than one bee visit per flower was necessary for commercial production and proposed that one colony per acre would be adequate to maximize fruit production. Many studies done in the past shows the importance of honeybees in crop production (Mann & Robinson, 1950; Mann, 1953; Nelson, 1964).

Table 3.11: Comparison of pollinators reported in the present study with previous publications.

	Pollinators		
Species	**Present study**	**Previous publication**	**Reference**
Oenothera biennis	Honeybees (*Apis mellifera*) #	Hawkmoths and *A. mellifera*	Hall *et al.* (1988)
Lantana camara	Thrips and butterflies	Thrips and butterflies	Mathur & Mohan Ram (1978)
Senna didymobotrya	*Xylocopa flavorufa*	*Xylocopa pubescens*	Dulberger (1981)
Psidium guajava	Honeybees	Honeybees	Purseglove (1968)

moth visitation was recently observed by S.D Johnson (personal communication).

Bee visits by *Xylocopa* bees in *Senna didymobotrya* and *Sesbania punicea* illustrated the importance of the buzz pollination system in removing pollen (>10 000 grains) and contributing to the reproductive success of these alien invasive plant species. *Senna didymobotrya* is also pollinated by *Xylocopa* bees insects in its country of origin (Table 3.11).

Examination of butterflies on *Lantana camara* showed that many species contribute to fruiting success, although pollen loads per individual were low (Table 3.7). There is a strong association of thrips with *Lantana camara* and *Solanum mauritianum*. In India *L. camara* is also visited by thrips and butterflies species (Table 3.11).

The build-up of the thrip population is reported to encourage movement within the flower, which eventually promotes self-pollination (Mathur & Mohan Ram, 1986). Thrips were found to be effective pollinators of cross-pollinated *Popowia pisocarpa* (Annonaceae) in the lowland Dipterocarp forest in Sarawak (Momose *et al.* 1998). Deposition of pollen by thrips onto the stigma of a flower was observed in a laboratory study of association of thrips with yellow flowers in *Lantana camara* by Annand (1926). This is confirmed in the present study which shows high levels of fruit set in *L. camara* flowers exposed to thrips, compared to flowers from which thrips were excluded (Fig. 3.23).

In this study, alien invasive species were mostly pollinated by common visitors such as honeybees, butterflies, hawkmoth and thrips. Results from this study show that 71% of the 14 alien species for which pollinators were obtained were pollinated by honeybees. This generalization and flexibility in pollination systems is essential to compensate for the lack of pollinators from the native habitat. For example, *O. biennis* and *S. didymobotrya* are pollinated by different, yet similar pollinators to those in their native environment (Table 3.11). In the case of *Ipomoea alba*, pollination was carried out by the large hawkmoth *Agrius convolvuli*. Invasion of alien plants in KwaZulu Natal is attributable in part to a generalised pollination system, as proposed by Baker (1955).

Chapter 4

Patterns and ecological determinants of fruit set

Abstract

High levels of natural fruit set (median=71.5%) were found in 19 alien invasive plant species sampled in South Africa. High levels of fecundity were attributed to high rates of visitation by honeybees and/ or fully autogamous breeding systems. There was no indication that pollen availability is a limiting factor for reproductive output in these alien invasive plants as hand pollination did not lead to increased fruit set in five species. Thus, fruit set in these alien species is probably at the maximum possible in terms of their available physiological resources.

Introduction

Supplemental hand pollination experiments have been regarded as an integral tool in recent studies that seek to determine limiting factors for plant reproductive success (Burd, 1999; Larson & Barrett, 2000). The effect of pollen limitation on reproductive output has rarely been examined in alien invasive species. Species introduced into a new habitat may suffer from lack of pollination service (Parker, 1997). If colonizing species are pollen limited, the ability of plants to colonize new areas may be affected by the availability of pollinators. If a plant has a specialized pollination system and the pollinator is absent in the new environment, pollen limitation may limit seed production and the success of the plant species in the new environment.

Pollen limitation occurs when the production of seed is less than the maximum seed potential, because of the inadequate numbers of pollen grains to fertilize the ovules (Bierzychudek, 1981; Fox, 1992; Ramsey, 1995). Although there is evidence to suggest that fruit and seed production are often limited by resources (Zimmerman & Pyke, 1988; Haig & Westoby, 1987), recent studies have shown that pollen availability frequently limits, fruit and seed production not only on individual flowers,

but also on the plant as a whole (Ehrlin, 1992; Campbell & Halama, 1993; Burd, 1994; Larson & Barret, 2000).

A relatively small number of studies have been directed towards the examination of pollen limitation in colonizing species. The prediction is that species that are not autogamous and that rely heavily on pollination service are more likely to be limited by pollen availability. The aim of this study was to document levels of fruit and seed set in alien invasive plants and to determine whether insufficient pollen limits the reproductive success of these plants.

Materials and methods

Natural levels of fruit set were determined for 19 alien invader plant species. To ensure that site by site variation was accounted for, fruit set was measured from three different sites for each species. Fruiting success was measured as a percentage of the original number of flowers per inflorescence.

Pollen limitation experiments were conducted on *Canna indica*, *Caesalpinia decapetala*, *Lilium formosanum*, *Oenothera biennis* and *Senna didymobotrya*. Flowers were hand pollinated with pollen collected at least 10m away from the experimental plant. Hand pollination is the addition of pollen to the stigma of another flower without bagging or other pollinator exclusion technique. Hand-pollination should be undertaken on all the flowers on a plant, so that an increase in seed set in one flower is not compensated for by a decrease in seed set of another flower on the same plant (Campbell & Halama, 1993). If it is not possible to hand pollinate all flowers within a plant, it is then necessary to have two sets of controls, one adjacent to hand pollinated flowers and another on a different plant. If there is no difference in fruit and seed set between these controls, then resource shunting is unlikely to have occurred (Zimmermann & Pyke, 1988).

In *Canna indica* and *Oenothera biennis*, flowers were hand pollinated and compared with adjacent unmanipulated control flowers and unmanipulated flowers on a different plant. In *Senna didymobotrya*, supplemental outcross pollen was added to the stigmas of flowers on 30 inflorescence

stems. All flowers on the inflorescence were hand pollinated with additional pollen and compared with the control flowers of another inflorescence.

Forty individual plants were marked for the hand pollination experiment in *Lilium formosanum* and forty were marked as controls. Flowers (n=121) were hand pollinated and flowers (n=119) on control plants were left unmanipulated. Newly opened flowers were checked each day so that all the flowers on a plant were hand pollinated, to avoid a situation where supplementation of a hand pollinated subset of flowers may boost reproductive output at the expense of other flowers on the same plant (Johnston, 1991). Flowers opposite to the experimental flowers were marked to test if the flowers were not limited by resources in *Oenothera biennis*, *Canna indica*, *Senna didymobotrya* and *Caesalpinia decapetala*. Fruits and seeds were collected, weighed and counted.

Data analysis

Treatments were compared using one-way ANOVA followed by the Tukey multiple range test (Zar, 1996).

Results

Natural fruit set

Natural levels of fruit set varied from 5.1 % to 99 % amongst the nineteen alien invasive species with a median of 71.5% (Fig 4.26). The fruit set of each species will be discussed below. Results indicate that in the 5 species that were hand pollinated, there was no significant difference between treatments and hence reproduction in these alien invasive species is not pollen limited.

Canna indica

Supplementation of pollen on stigmas had no significant effect on fruit set between hand pollination, opposite control and control treatments (Fig. 4.27A). There was no significant difference in mean fruit mass per flower (Fig. 4.27B), seed mass per fruit (Fig.4.27C) or the number of seeds produced (Fig 4.27 D) between the three treatments, which indicates that *Canna indica* is not pollen limited.

Caesalpinia decapetala

Supplementation of pollen on the stigma had no significant effect on fruit set, mean fruit mass per flower, seed mass per fruit or the number of seeds produced (Fig 4.28 A-D).

Lilium formosanum

There was no significant difference in percentage fruit set between the natural pollinated plants (controls) and hand-pollinated plants (Fig 4.29 A). There was also no significant difference in fruit mass per flower, seed mass, or the number of seeds per fruit (Fig 4.29 C-D).

Senna didymobotrya

Hand-pollination had no significant effect on fruit set, mean fruit mass per flower, seed mass per fruit or the number of seeds produced per fruit (Fig 4.30 A-D).

Oenothera biennis

There was high percentage of fruit set in both hand-pollinated and control flowers. Supplementation of pollen on the stigma had no significant effect on fruit set, mean fruit mass per flower, seed mass per fruit or number of seeds per fruit (Fig 4.31 A-D).

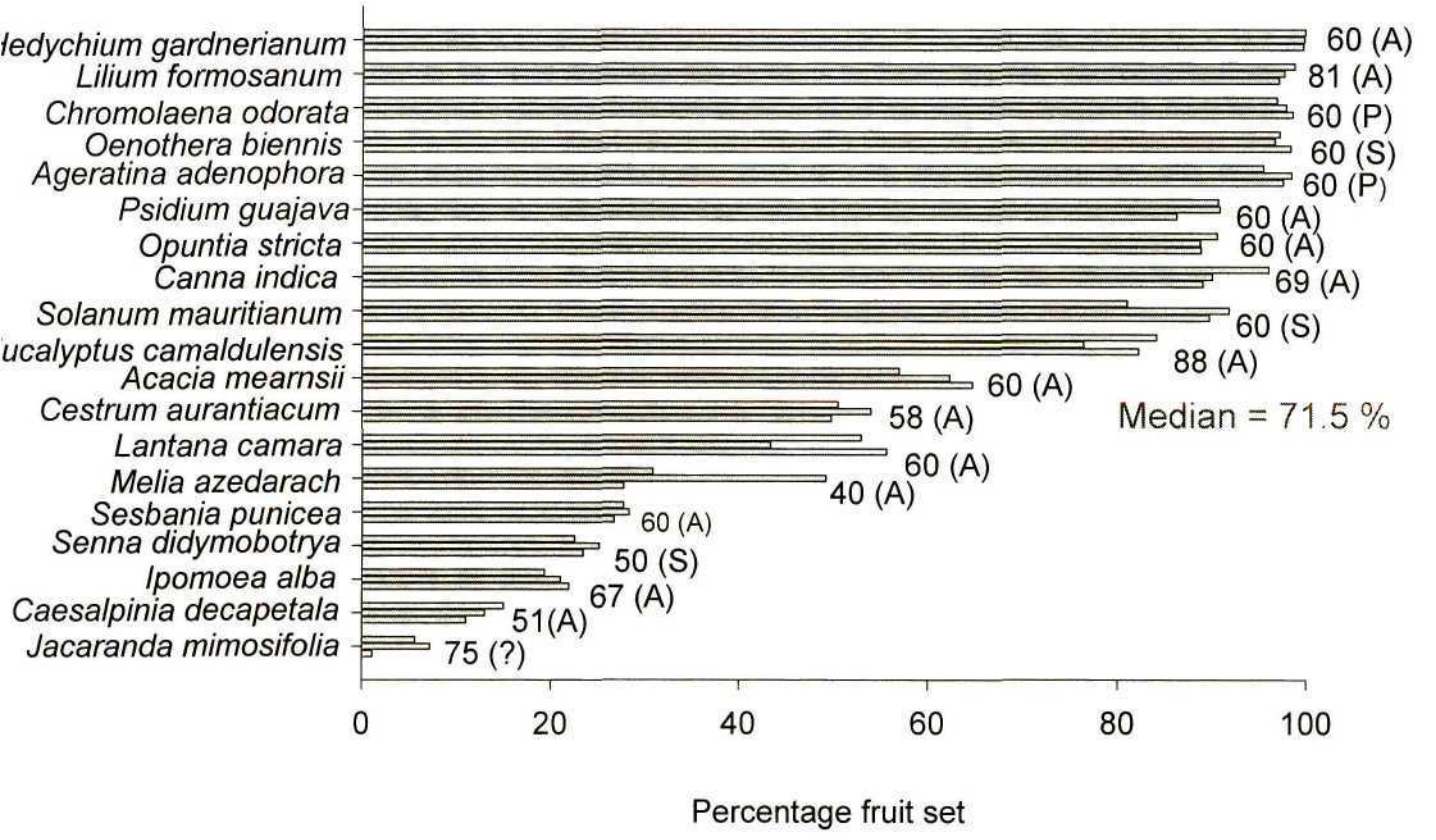

Figure 4.26. Natural levels of fruit set in 19 alien invasive species and one non-invasive species (*Cassia multijuga*). A=autogamy, P=apomictic, S=self-compatible and ?= unknown breeding systems. The three bars in each species represent three different study sites. The numbers on the right hand side of the bars graph represent the number of flowers for each treatment.

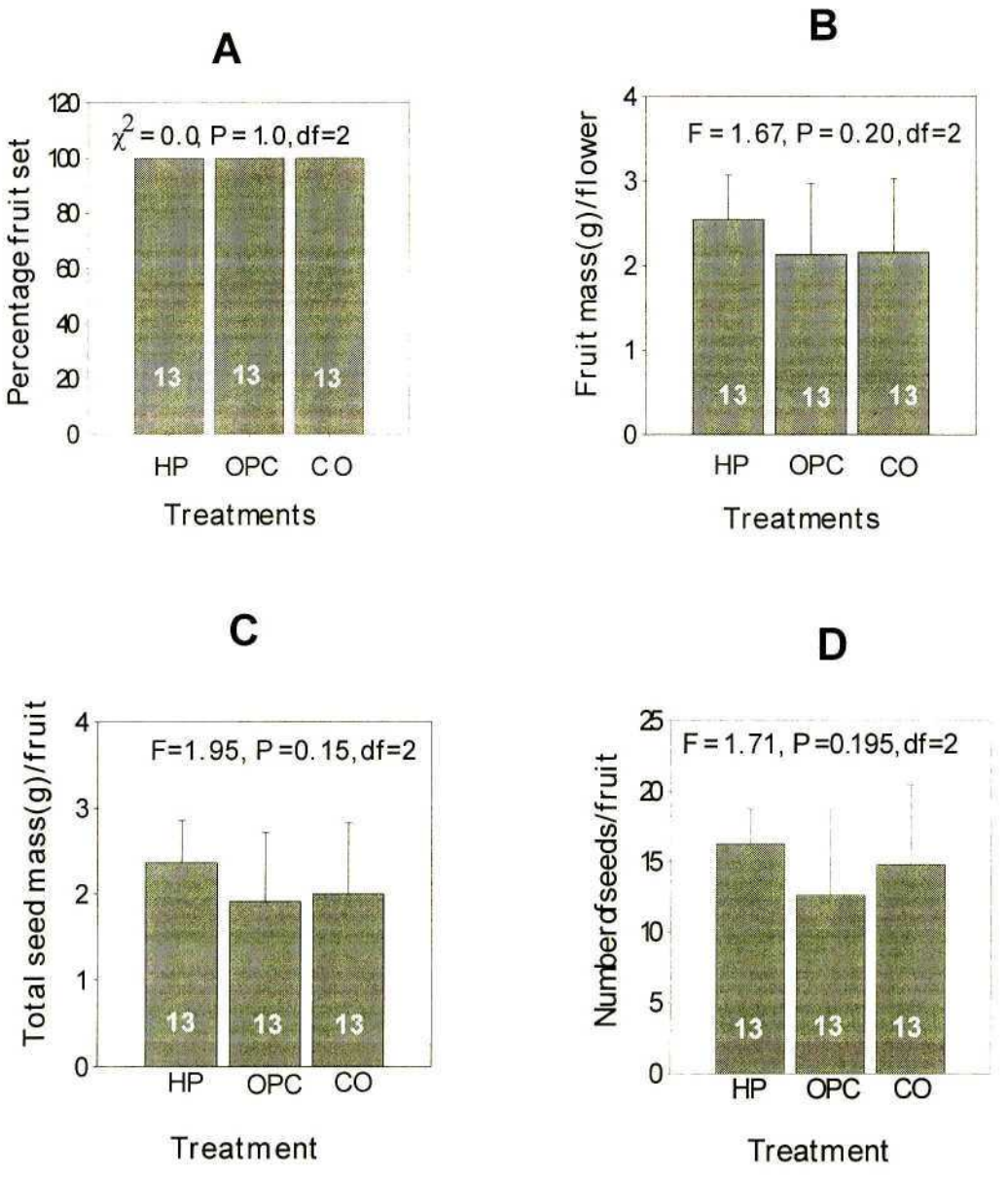

Figure 4.27. Pollen limitation experiment results for ***Canna indica.*** A. Percentage fruit set (n=number of flowers). B. Fruit mass per flower (n=number of fruits). C. Seed mass per fruit (n=number of fruits). D. Number of seeds per fruit (n=number of fruits). HP = hand pollination, OPC = opposite control (adjacent flower) and CO = control (on a separate plant). Bars represent means and error bars represent the standard deviation.

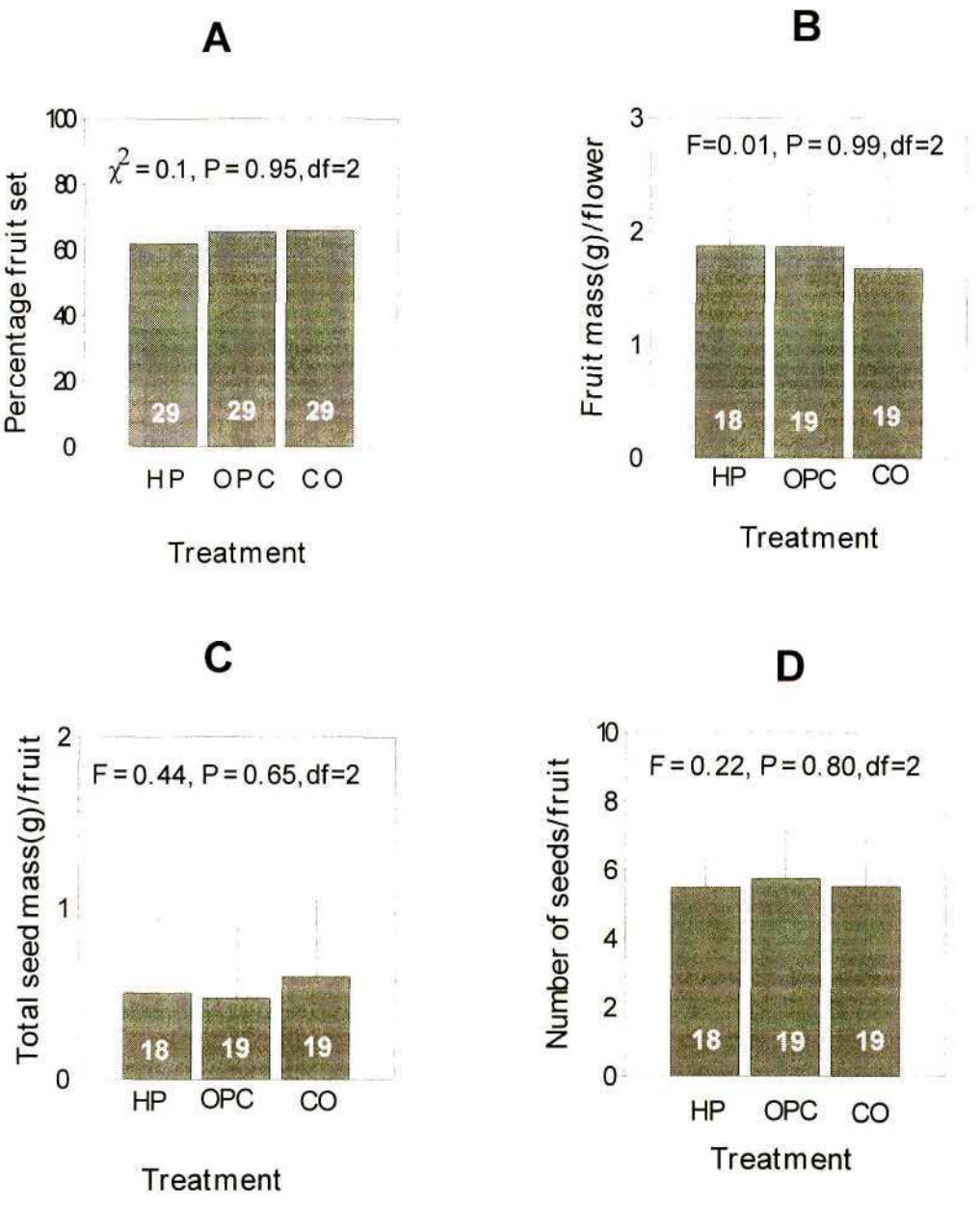

Figure 4.28. Pollen limitation results for ***Caesalpinia decapetala***. A. Percentage fruit set (n=number of flowers). B. Fruit mass per flower (n=number of fruits). C. Seed mass per fruit (n=number of fruits). D. Number of seeds per fruit (n=number of fruits). HP = hand pollination, OPC = opposite control and CO = control. Bars represent means and error bars represent the standard deviation.

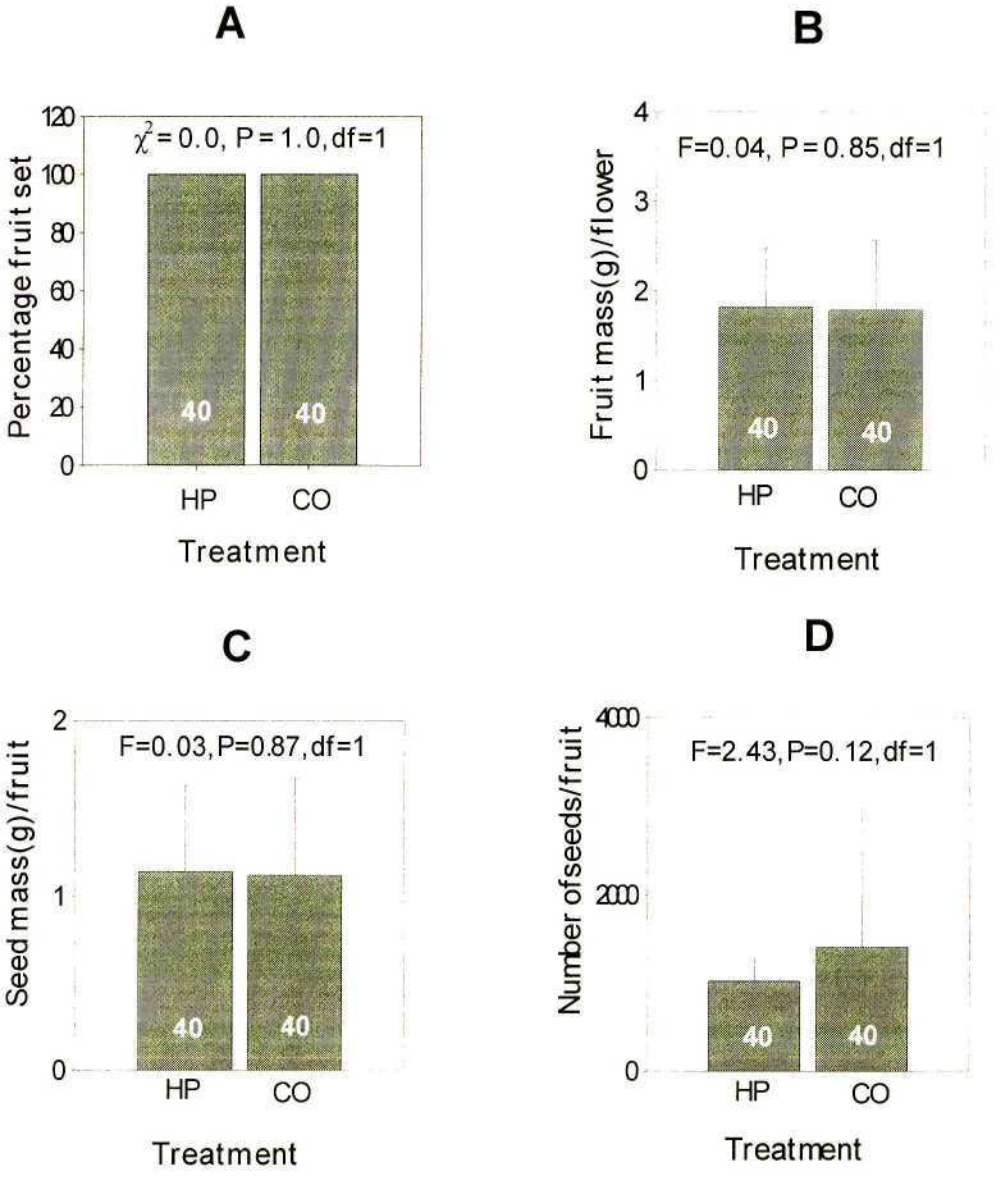

Figure 4.29. Pollen limitation results for ***Lilium formosanum***. A. Percentage fruit set (n=number of flowers). B. Fruit mass per flower (n=number of fruits). C. Seed mass per fruit (n=number of fruits). D. Number of seeds per fruit (n=number of fruits). HP = hand pollination, OPC = opposite control and CO = control. Bars represent means and error bars represent standard deviation. Numbers inside the bars represent the sample size.

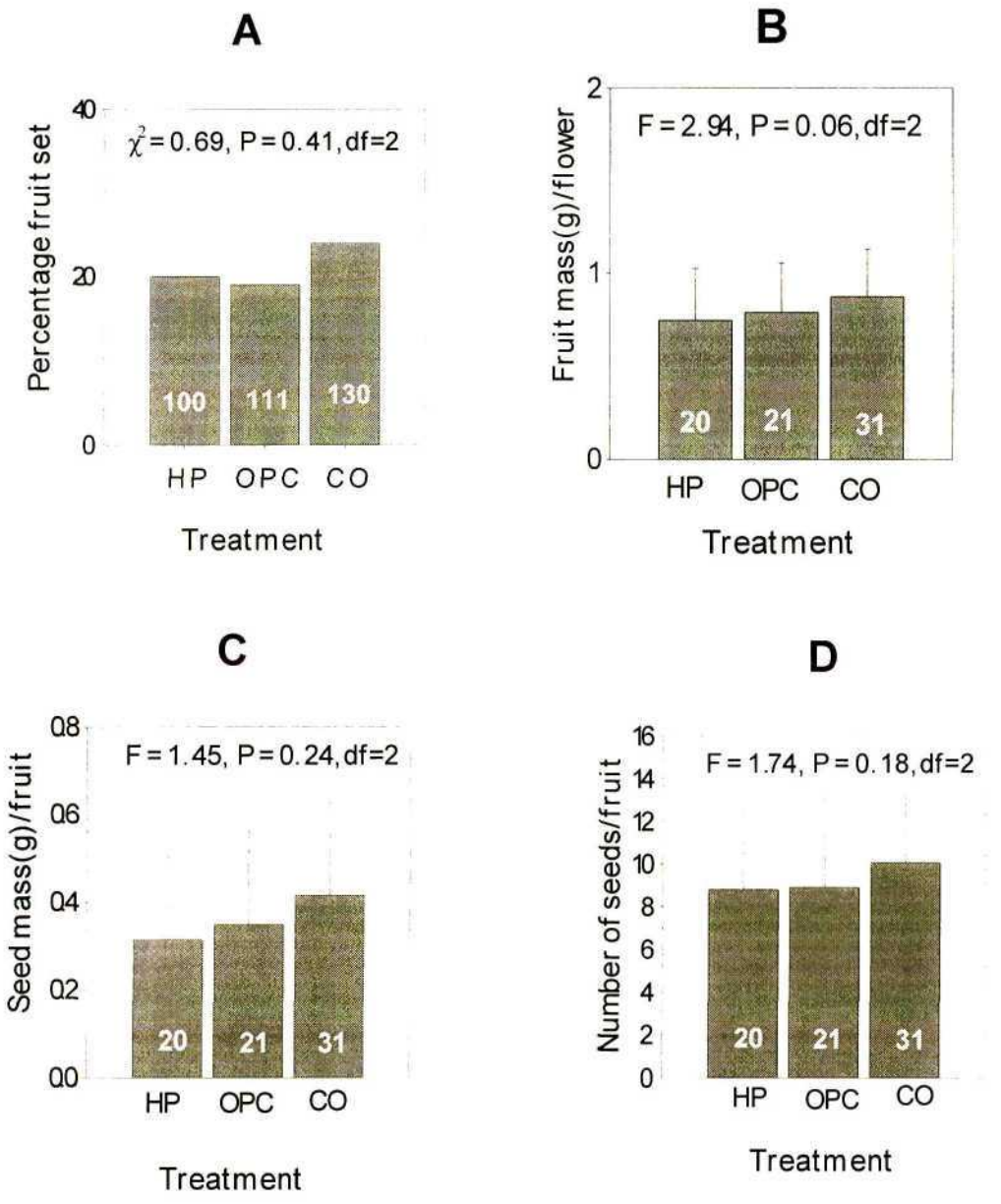

Figure 4.30. Pollen limitation results for ***Senna didymobotrya***. A. Percentage fruit set (n=number of flowers). B. Fruit mass per flower (n=number of fruits). C. Seed mass per fruit (n=number of fruits). D. Number of seeds per fruit (n=number fruits). HP = hand pollination, OPC = opposite control and CO = control. Bars represent means and error bars represent standard deviation. Number inside the bars represent the sample size.

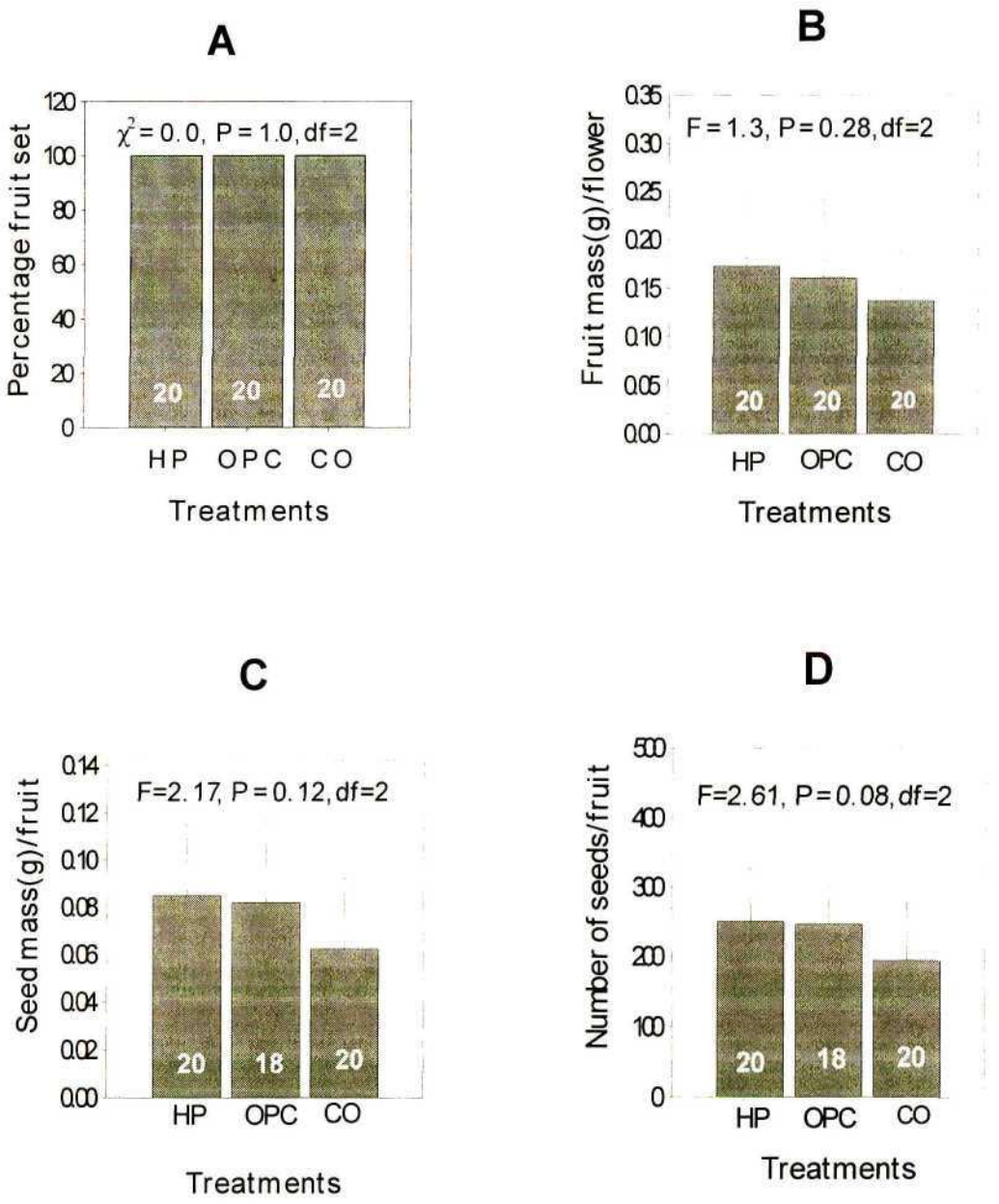

Figure 4.31. Pollen limitation results for ***Oenothera biennis.*** A. Percentage fruit set (n=number of flowers). B. Fruit mass per flower (n=number of fruits). C. Seed mass per fruit (n=number of fruits). D. Number of seeds per fruit)(n=number of fruits). HP = hand pollination, OPC = opposite control and CO =control. Bars represent means and error bars represent standard deviation. The number inside the bars represent the sample size.

Discussion

Alien invasive plant species appear to have naturally high levels of fruit set (Fig 4.26). Stigmas appeared to be saturated with pollen deposited through autogamy or by insects, as supplemental hand-pollination did not increase fruit and seed set in the five sampled species. These results of supplemental hand pollination on *Lilium formosanum*, *Caesalpinia decapetala* and *Canna indica* confirm the expectation that reproduction in autogamous species are generally not limited by pollen availability. However, *Oenothera biennis* and *Senna didymobotrya* are not fully autogamous (Table 2.5) and rely on high levels of pollination service.

Evidence for pollen limitation

There is no evidence that these five alien invasive plant species suffer a reproductive loss due to pollen limitation. Previous studies have shown that low pollination service reduces fruit set markedly (Schemske *et al.* 1978; Bierzychudek, 1981). In such instances, allogamous plants will be disadvantaged, because of lack of pollen vectors. Previous research has focused on self-incompatible or facultatively outcrossing species in their native habitat (Richardson *et al.* 2000) with no studies of pollen limitation in alien invasive plant species. There was no sign of pollen limitation in these five alien invasive species, Parker (1997) reported evidence of pollen limitation in Scotch broom (*Cytisus scoparius*), an invasive species in North America. Parker (1997) found that the reproductive consequences of pollen limitation was less in urban populations where there was a high number of bumblebees and when the flowers bloomed simultaneously compared to prairie populations where plants flowered asynchronously with bumblebee availability.

Pollination limitation in shrubby invasive species

Large plants such as *Caesalpinia decapetala* and *Senna didymobotrya* produce large numbers of flowers but a limited number of fruits. Many flowers in such cases are aborted. Resources seem to be another limiting factor for fruit development, as there was no evidence to suggest that pollen limits the reproductive success of *C. decapetala* and *S. didymobotrya*.

According to Lawrence (1993), the reproductive success of large plants is affected simultaneously by pollen and resource limitation because plants have to strike a balance between producing seeds and maintaining growth. Lawrence (1993) suggested that success in reproduction may be affected by allocation of the resources to different functional areas of the plant. Allocation of resources seems to be skewed to vegetative growth in *C. decapetala* and less energy is allocated to flower development and seed maturation. *Caesalpinia decapetala* and *Chromolaena odorata* can reproduce vegetatively into a thick tangle of bushy shrubs that can occupy a large area.

High levels of natural fruit set in the five alien invasive plant species and absence of pollen limitation in these species can be attributed predominantly to their autogamous breeding systems. Thus, Baker's (1955) prediction that autogamous breeding systems in colonizing species frees plants from dependency on pollen vectors is supported.

Chapter 5

The role of reproductive biology in plant invasions

Abstract

This study of breeding and pollination systems of alien plants in KwaZulu-Natal provides strong support for Baker's (1955) rule. Autogamy was found in 72% of species sampled, which differs significantly from a general world flora in which only 14% of the species were autogamous. Flowers of alien plants mostly attract generalist honeybees, although a few show pre-adaptation to more specialized visitors such as hawkmoths, butterflies and *Xylocopa* bees. Thus alien invasive plants seem to possess a set of reproductive traits that promote high levels of fruit set (71.5%) in the study species.

Introduction

The role of reproductive biology in plant colonization has been widely discussed (Stebbins, 1950; Baker, 1955; Baker & Stebbins, 1965; Barret, 1996, Richardson *et al.* 2000), although there have been only a few surveys of reproductive traits in invasive plant species. Autogamy and self-compatibility are the dominant breeding systems in alien invasive plants in KwaZulu-Natal (Chapter 2), and these breeding systems were proposed to enhance invasion following long distance dispersal by Baker (1955).

Baker's (1955) initial hypothesis of the success of self-compatible species after long distance dispersal was prompted by observations of colonizing *Notostraca* invertebrates by Longhurst (1955). Baker (1955) observed that notostracans were ancestrally dioecious and that autogamous hermaphroditism was secondarily selected through long distance dispersal from obligate outcrossers. Baker (1955) also noted a high incidence of self-compatibility in the floras of the Galapagos Islands (Chapter 1). Previous studies have also documented the evolution of self-compatibility from self-

incompatible species (Carr *et al.* 1986; McMullen, 1987; Webb & Kelly, 1993).

Comparison and analysis of breeding systems

Breeding systems have received much attention from previous workers. East (1940) reviewed the occurrence of self-incompatibility in different orders of plants and Fryxell (1957) compiled a summary of more than 1000 species from different genera and some 123 families, giving each species a designation on a schematic triangle. According to the triangle model: 14 % of species are autogamous, 8 % are self-compatible, 57 % are self-incompatible and 21 % apomictic (Figure 5.32, Fryxell, 1957). Thus the number of self-incompatible (outcrossing) species in the general flora is higher than the number of autogamous and apomictic species combined (Fig. 5.32B).

In comparison, the 72 % of the 18 alien invasive species sampled in this study are autogamous, 17 % self-compatible, 0 % self-incompatible and 11 % apomictic (Fig 5.32B). This demonstrates that the number of autogamous species is significantly higher among alien invasive species than in the general flora (Fig. 5.32 A&B).

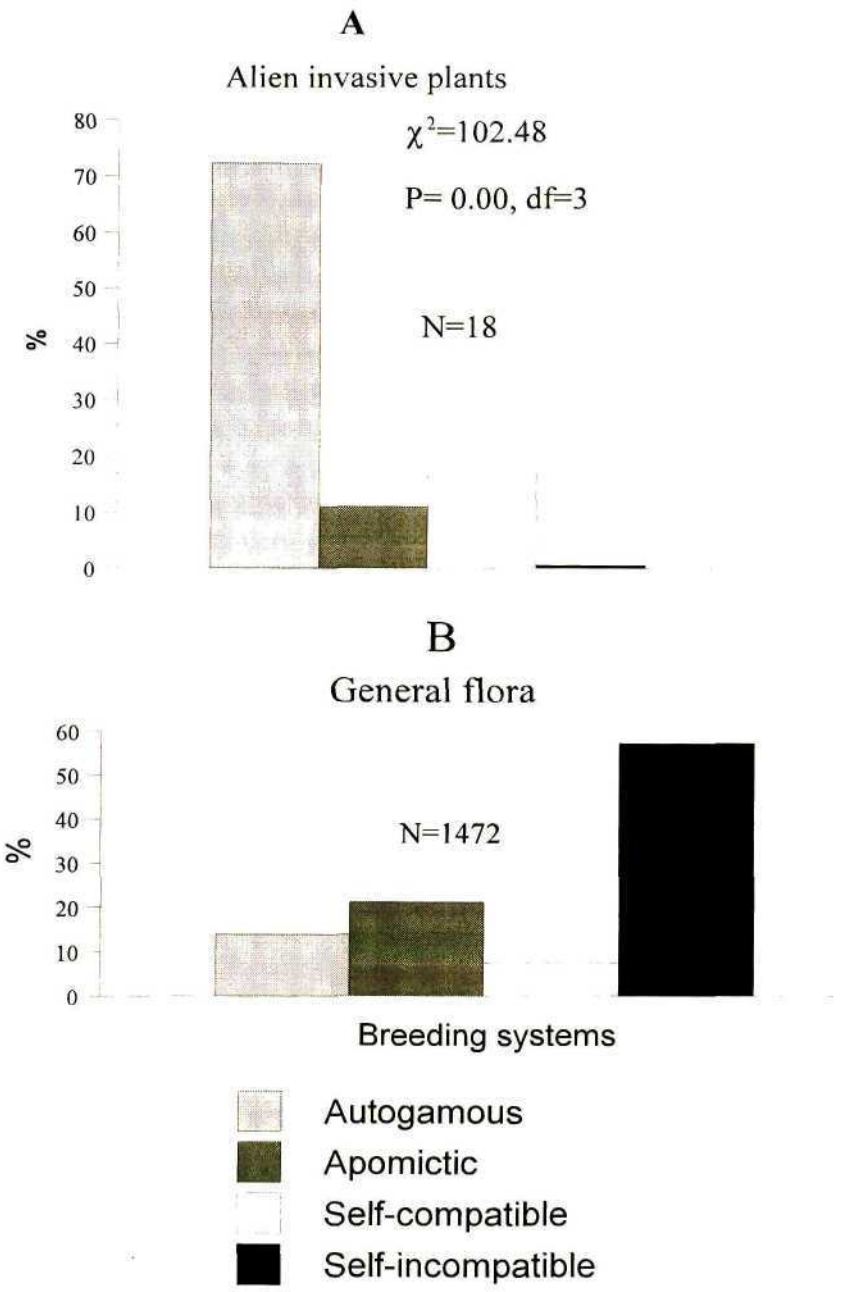

Figure 5.32. Pattern of breeding systems in alien invasive plants in South Africa (A) compared with a general flora (B) (from Fryxell's (1957) paper on modes of reproduction in higher plants).

These breeding system results illustrate the dominance of selfing in alien invasive plant species. Selfing is highlighted as a strategy that overcomes biotic barriers to invasion (Fig. 5.32). Richardson *et al.* (2000) cited a number of studies of autogamous alien plant species by other authors; the include Hottentot fig (*Carpobrotus edulis*) in California (Vilá *et al.* 1998); *Hypericum perforatum* in North America (Crompton *et al.* 1988) and Garlic Mustard (*Alliaria petiolla*) in North America (Cavers *et al.* 1979). Although there is a tendency for invasive species to be autogamous, there are also examples cases of outcrossing species that are invasive; Richardson *et al.* (2000) cited Purple Loosestrife, *Lythrum salicaria* (Levin 1970; Mal *et al.* 1992) and *Centaurea diffusa* and *C. maculosa* (Harrod & Taylor, 1995), both North American examples.

Despite the pattern of the results presented in Fig 5.32, the data presented here is limited to a small number of alien invasive species from KwaZulu Natal. Whether this kind of trend might be found generally in alien invasive species, as has been indicated by other authors (Baker, 1955; Price & Jain, 1981; Pannell & Barrett, 1998; Richardson *et al.* 2000) remains to be examined.

Apomictic species are common among flowering plants and they have been recorded in over 40 families around the world (Fryxell, 1957; Gupta *et al.* 1996; Calzada *et al.* 1996). Apomixis has been recorded most often among weedy species (Stebbins, 1974; Selbo & Carmichael, 1999). Success of apomictic species as colonizers has been recorded for *Chromolaena odorata* and *Ageratina adenophorum* in this study. One of the main advantages of apomictic asexuality is that genetic variation between individuals in the gene pool remains fixed (Richards, 1997). This maximises fitness and allows the species to fill a maximum number of niches in the environment (Dawkins, 1976). The main disadvantage is "the inability to recombine novel advantageous mutants that allow current evolution in the face of environmental changes, such as evolution by sexual competitors" (Richards, 1997).

Autogamy and asexual reproduction by apomixis are advantageous features that can enhance the spread of alien plants (Baker, 1965). Baker (1965) reported that *Tridax procumbers* from the American tropics had a combination of characteristics (rapid autogamous seed production and creeping vegetative growth), that enables this species to hit what has been called "*the weed jackpot*", because of its ability to spread using both characteristics.

The role of mutualisms in plant invasions

Many alien invasive plant species have overcome the barriers of establishment in new sites by forming mutualistic interactions between plants and pollinators (Richardson *et al.* 2000). In the past, research has focussed on biotic interactions that affect alien invasive species negatively (Richardson *et al.* 2000), and little on positive interactions that promote co-existence of plants and pollinators such as pollination (Bertness & Callaway, 1994; Callaway & Walker, 1997).

A major thrust of this discussion is the interaction between plants and their pollinators that results in greater invasiveness. It is evident that alien invasive plant species are served by a less diverse group of insect pollinators (Chapter 3). Honeybees are prevalent as visitors to most species, with a few species served by butterflies, hawkmoth and thrips.

A number of studies illustrate the role of mutualistic plant-pollinator interactions in plant colonization (Callaway & Walker, 1997; Vilá *et al.* 1998 & Levin, 1970). The interaction of alien invasive plants with their pollinators was first noted by Darwin (1876): who observed that two years after planting the shrub *Berberis dulcis* the flowers were visited by bees (Richardson *et al.* 2000). Another observation by Darwin, cited by Richardson *et al.* (2000) is that *Impatiens capensis* was visited by native bumble bees in England after its introduction.

Paton (1993) in his study of honeybee interactions with the Australian flora showed that a large number of plants were used as a source of nectar and pollen by honeybees. Mahy *et al.* (1998), in a study of the reproductive biology of *Calluna vulgaris*, found that honeybees were far more effective

pollinators than bumblebees. Introduced honeybees (*Apis mellifera*) were the most common pollinator of *Nemophila* in cultivated areas (Cruden, 1972).

In this study, *Oenothera biennis* and *Solanum mauritianum* were observed to occur in disturbed sites and are visited intensively by honeybees (Chapter 3). It is important to understand these positive interactions between plants and their pollinators in order to be able to predict the invasiveness of alien species brought about by these interactions (Richardson *et al.* 2000).

Generalization versus specialization

According to Baker (1955) 's rule, a generalized pollination system is expected in colonizing species (Johnson & Steiner, 2000). Pollination systems are generalized when flowers attracts many different species of insects or when pollinators are generalist, such as honeybees.

When alien invasive plant species arrive in new localities where the original pollinators are no longer available, they often subscribe to the available common visitors (Baker, 1965). In this study, generalization of pollination system was predominant among alien invasive plant species. Honeybees played an important role in the fruiting success of *Solanum mauritianum* and *Oenothera biennis*. *Oenothera biennis* was visited by hawkmoths and bees in its native environment (Hall *et al.* 1988), and in South Africa this species attracts honeybees and moths.

Pre-adaptation in a specialized pollination system is also evidenced in *Senna didymobotrya* which is visited by different *Xylocopa* bees in South Africa and its natural habitat (Dulberger, 1981). Thus flexibility in the pollination systems of alien invasive plant species is important in order to colonize new sites.

Predicting invasiveness

Predicting the invasiveness of alien species encompasses many factors. This study shows that breeding systems can be used as a mechanism of screening plants. High levels of seed set in bagged plants are a warning signal for the possibility of further invasiveness. High seed set in bagged flowers would indicate that an autogamous species can impose itself by spreading rapidly in the new environment. Potential invasive plants can be tested by bagging plants in the green house or field environment for high levels of seed set (explained in Chapter 2).

Pollination systems can be screened by introducing alien plants in a greenhouse or field environment to see whether common pollinators such as honeybees are able to pollinate their flowers. Such mutual relationships will suggest the possibility of further invasion. Plant species coming from another continent may be released from pollination deficiency when successfully attracts generalist pollinators such as honeybees.

Autogamy and pollination by honeybees appear to be the two traits that are clearly associated with invasiveness in this study. The species highlighted here have mostly spread out of control, but if their reproductive attributes had been known before introduction to South Africa, it might have been possible to ban their import. Screening protocols for introduced species that include experimental investigation of breeding and pollination systems, may help us to predict whether a particular plant species has the potential to invade natural ecosystems.

There are already laws that prevent the transportation of declared weedy species in South Africa. According to the Conservation of Agricultural Resource Act no. 43 of 1983 - Prohibition of the Spread of Weedy Species:

"1(a) No person shall- sell, agree to sell or offer, advertise, keep, exhibit transmit, send , convey or deliver for sale, or exchange for anything or dispose of to any person in any manner for a consideration, any weed or

(b) in any other manner whatsoever disperse or cause or permit the dispersal of any weed from

any place in the Republic to any other place in the Republic.

The law is less specific about plants which are not yet invasive, but have the potential to become so. There is little concern about the protocols to be followed when importing plants from overseas or exporting South African plants to other countries. The National Botanical Institute is a prime offender with a seed catalogue that numbers almost a thousand species, some of which may join the growing list of South African plants, such as *Chrysanthemoides monilifera*, that have become major weeds in other countries. A first step would be to remove species from the list which show weedy tendencies even in South Africa, such as colonization of road margins.

The porosity of plant movement allowed by authorities and management structures such as nurseries and botanical gardens can lead to a situation where some plants end up in the country before being screened for their potential weediness.

Recommendations

There is consensus internationally that invasion by alien invader plant species is one of the greatest threats to bio-diversity (Everett, 2000). Emphasis has been put on chemical control and bio-control without studying the ecology of the species in general. There are attributes that contribute to successful invasion such as high seed output in reproductive success. Pollination systems play an important role in the reproductive success of a species.

Species that are already established, problematic and threatening further colonization must be monitored to prevent further invasion. The problem is how we can find agents to reduce the spread of alien invasive plants when they continue to produce high seed output. Action must be taken to reduce flower production, or to prevent fertilization from taking place, e.g. by introducing galling insects that specialize on flower buds and reduce reproduction, as in *Acacia longifolia* (Dennill *et al.* 1999).

There has been little concern as to what type of plants are brought into South Africa. This has resulted in unwanted plants becoming widespread and costing South Africa millions of rands per year. Due to the minimal restrictions, very dangerous plants have been introduced, causing an enormous impact on our ecosystems. Amazingly, there are strong laws concerning the introduction of other species such as bio-control agents, but very little concern about plants. I propose that laws should be passed whereby plant species can be screened for breeding systems before being introduced into the country. Although there has been a multidimensional approach regarding eradication of alien invader species, there is little concern about their ecological aspects. It is imperative to take a holistic approach in order to prevent and eradicate alien plant invasions.

Appendices

Appendix 1. Voucher information of the collected insects.

Plant species	Insect species (number of species)	Locality (around Pietermaritzburg (PMB) in South Africa)	Date	Collector
Acacia mearnsii	*Apis mellifera* (10) (Apidae)	*Ferncliffe* nature Reserve	*20/07/00*	***T D Rambuda***
Caesalpina decapetala	*Apis mellifera* (23) (Apidae)	*Ferncliffe nature Reserve*	*26/08/99*	***T D Rambuda***
Caesalpina decapetala	*Xylocopa flavorufa* (10) (Anthophoridae)	*Ferncliffe nature Reserve*	*03/09/99-27/10/99*	***T D Rambuda***
Jacaranda mimosifolia	*Apis mellifera* (20) (Apidae)	*Scottsville*	*17/11/99*	***T D Rambuda***
Hedychium gardnerianum	*Apis mellifera* (11) (Apidae)	*World's View*	*01/02/00*	***T D Rambuda***
Eucalyptus camaldulensis	*Apis mellifera* (10) (Apidae)	*Ferncliffe nature Reserve*	*10/05/99*	***T D Rambuda***
Oenothera biennis	*Apis mellifera* (10) (Apidae)	*Hilton Wetland Area*	*19/02/99*	***T D Rambuda***
Opuntia stricta	*Apis mellifera* (10) (Apidae)	*Ashburton*	*20/12/99*	***T D Rambuda***
Sesbania punicea	*Apis mellifera* (10) (Apidae)	*Scottsville*	*01/12/99*	***T D Rambuda***
Sesbania punicea	*Xylocopa flavorufa* (10) (Anthophoridae)	*Scottsville*	*25/11/99-02/12/99*	***T D Rambuda***
Solanum mauritianum	*Apis mellifera* (10) (Apidae)	*Ferncliffe nature Reserve*	*11/05/99-26/05/99*	***T D Rambuda***
Senna didymobotrya	*Apis mellifera* (10) *(Apis mellifera)*	*Hayfields*	*27/07/00-05/08/00*	***T D Rambuda***
Senna didymobotrya	*Xylocopa flavorufa* (20) (Anthophoridae)	*Hayfields*	*27/07/00*	***T D Rambuda***
Ipomoea alba	*Agrius convolvuli* (7) (Sphingidae)	*Scottsville*	*18/02/00-07/03/00*	***T D Rambuda***
Lantana camara	*Acraea agloanice* (3) (Acraeidae)	*Ferncliffe nature Reserve*	*27/04/99-30/04/99*	***T D Rambuda***
Lantana camara	*Acraea cabira* (3) (Acraeidae)	*Ferncliffe nature Reserve*	*27/04/99-30/04/99*	***T D Rambuda***
Lantana camara	*Acraea natalica* (10) (Acraeidae)	*Ferncliffe nature Reserve*	*21/04/99-27/04/99*	***T D Rambuda***
Lantana camara	*Amauris echeria (8)* *(Nymphalidae)*	*Ferncliffe nature Reserve*	*26/04/99-30/04/99*	***T D Rambuda***
Lantana camara	*Catopsilia florella (4)* *(Pieridae)*	*Ferncliffe nature Reserve*	*01/05/99-02/05/99*	***T D Rambuda***
Lantana camara	*Coenyropsis natalii (10)* *(Satyridae)*	*Ferncliffe nature Reserve*	*01/05/99-02/05/99*	***T D Rambuda***
Lantana camara	*Danaus chrysippus (4)* *(Nymphalidae)*	*Ferncliffe nature Reserve*	*27/04/99-30/04/99*	***T D Rambuda***

Lantana camara	*Eurema brigitta (5) (Pieridae)*	*Ferncliffe nature Reserve*	*30/04/99*	***T D Rambuda***
Lantana camara	*Papilio nireus (5) (Papilionidae)*	*Ferncliffe nature Reserve*	*10/06/98*	***T D Rambuda***
Lantana camara	*Papilio demodocus (3) (Papilionidae)*	*Ferncliffe nature Reserve*	*30/04/99*	***T D Rambuda***
Lantana camara	*Vanessa cardui (5) (Nymphalidae)*	Ferncliffe nature Reserve	*30/04/99-01/05/99*	***T D Rambuda***

Appendix 2. Voucher information of the collected alien plant invasive species.

Family	Plant species	Locality (around Pietermaritzburg (PMB) in South Africa)	Habitat	Date	Collector
Asteraceae	*Ageratina adenophora*	*Sweetwaters*	*Along road side, dry & moist*	*16/09/98*	***T. Rambuda***
Asteraceae	*Chromolaena odorata*	*Paradise Valley*	*Along roadside, moist habitat*	*23/06/99*	***T. Rambuda***
Bignoniaceae	*Jacaranda mimosifolia*	*Scottsville*	*Along roadside, dry moist habitat*	*17/11/99*	***T. Rambuda***
Cactaceae	*Opuntia stricta*	*Ashburton*	*Dry habitat*	*20/12/99*	***T. Rambuda***
Cannaceae	*Canna indica*	*Hilton*	*Moist habitat*	*23/03/99*	***T. Rambuda***
Convolvulaceae	*Ipomoea alba*	*Scottsville*	*Along riverbank, moist habitat*	*17/02/00*	***T. Rambuda***
Fabaceae	*Acacia mearnsii*	*Ferncliffe*	*Dry moist habitat*	*27/07/00*	***T. Rambuda***
Fabaceae	*Caesalpinia decapetala*	*Ferncliffe*	*Along road side, dry moist area*	*26/07/00*	***T. Rambuda***
Fabaceae	*Senna didymobotrya*	*Hayfields*	*Dry moist habitat*	*27/07/00*	***T. Rambuda***
Fabaceae	*Sesbania punicea*	*Scottsville*	*Along riverbank, moist habitat*	*02/12/99*	***T. Rambuda***
Liliaceae	*Lilium formosanum*	*World's View*	*Grassland patch surrounded by plantation, dry moist habitat*	*23/03/99*	***T. Rambuda***
Meliaceae	*Melia azedarach*	*Scottsville*	*Dry moist habitat*	*13/10/99*	***T. Rambuda***
Myrtaceae	*Eucalyptus camaldulensis*	*Ferncliffe*	*Dry moist habitat*	*13/10/99*	***T. Rambuda***
Myrtaceae	*Psidium guajava*	*Scottsville*	*Dry moist area*	*04/11/99*	***T. Rambuda***
Onagraceae	*Oenothera biennis*	*Hilton*	*Wetland area, moist habitat*	*23/03/99*	***T. Rambuda***
Solanaceae	*Cestrum aurantiacum*	*Ferncliffe*	*Disturbed site, dry moist habitat*	*13/10/99*	***T. Rambuda***
Solanaceae	*Solanum mauritianum*	*Ferncliffe*	*Surrounded by plantation sites, dry moist area*	*11/05/99*	***T. Rambuda***
Zingiberaceae	*Hedychium gardnerianum*	*World's View*	*Dry moist area*	*23/03/99*	***T. Rambuda***

Verbenaceae	*Lantana camara*	*Ferncliffe*	*Surrounded by plantation sites, dry moist habitat*	*23/03/99*	***T. Rambuda***

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